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Supporting Information

Experimental

UV Titration

The experiment was carried out in 100 mM NaCl, 50 mM sodium phosphate, pH 7.0. $d[AG_3(T_2AG_3)_3]$ was titrated (in 20 increments) into a solution of TMPyP4 (approx. 4 μM), at constant porphyrin concentration, until a 1:1 mixture of quadruplex and porphyrin was reached. The A_{420} (free porphyrin λ_{max}) was plotted as a function of the quadruplex:porphyrin ratio, Figure 5.

Job Mixing Curve

A mixing curve for TMPyP4 and $d[AG_3(T_2AG_3)_3]$ was determined by a variation on the method of reference 9. Two series of solutions (21 each) were prepared. In the sample solutions, the sum of the concentrations of TMPyP4 and $d[AG_3(T_2AG_3)_3]$ was constant (5 μM) and the mole fraction of TMPyP4 varied from 0 to 100 %. For each sample solution, 50 μl of 10 × PBS (100 mM NaCl, 50 mM sodium phosphate, pH 7.0) was added to 350 μl of distilled water. Then between 0 and 100 μl (with an increment of 5 μl) of 25 μM TMPyP4 stock solution was added as appropriate. The final volumes were adjusted to 500 μl with 25 μM DNA stock solution. The blank solutions were prepared similarly, the TMPyP4 concentration was the same as in the corresponding sample solution but the final volumes were adjusted to 500 μl with distilled water rather than with DNA stock solution. The spectrophotometric measurements were carried out in 1.0 cm cell at 430 nm. The A_{430} (λ_{max} of the bound porphyrin) data were plotted against the mole fractions of TMPyP4 and normalized by the published procedure[9] (Figure 6).

Photocleavage Assay

The 39-base single-strand DNA (5′-CATGGTGGTTTGGGTTAGGGTTAGGGTTAGG-GTTACCAC-3′) was synthesized on a Perseptive Biosystems Expedite nucleic acid synthesizer and purified by polyacrylamide gel electrophoresis. The DNA was labeled with ^{32}P at the 5′-end and stored in buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 3000 cpm/μl. For each photocleavage reaction, 10 μl of DNA (~5 ng) was mixed with 10 μl of 200 mM KCl, boiled for 10 min and cooled to room temperature. For the control experiments, 10 μl of distilled water was added instead of the KCl solution. The mixtures were transferred to a 96 well plate, 2 μl of 1 μM aqueous TMPyP4 solution added and the samples exposed to a 24 W fluorescent day-light under a glass filter for various periods of time. The reactions were stopped with 100 μl of calf thymus DNA (0.1 μg/μl) and after phenol-chloroform extraction, the samples were subjected to strand breakage treatment and ethanol precipitation (Han, F. X.; Hurley, L. H. *Biochemistry* **1996**, *35*, 7993–8001). The DNA samples were loaded onto a 12% polyacrylamide gel for electrophoresis and visualized using a phosphorimager (Molecular Dynamics, model 445 S1).

DNA Synthesis Arrest Assay

This assay was a modification of that described by Weitzmann et al.[10] Briefly, primers (24 nM) labeled with [γ-^{32}P]ATP were mixed with template DNA PQ74 (12 nM) in a Tris-HCl buffer (10 mM Tris, pH 8.0) containing 10 mM $MgCl_2$ and heated at 90 °C for 4 min. After cooling at room temperature for 15 min, spermidine (to give 100 μM) and TMPyP4 (to give the concentrations indicated in the figure) were added. The primer extension reactions were initiated by adding dNTP (final concentration 100 μM) and Taq DNA polymerase (2.5 U/reaction, Boehringer Mannheim). The reactions were incubated at 55 °C (or otherwise as indicated) for 15 min, then stopped by adding an equal volume of stop buffer (95% formamide, 10 mM EDTA, 10 mM NaOH, 0.1% xylene cyanol, 0.1%

bromophenol blue). The products were separated on a 12% polyacrylamide sequencing gel. The gels were then dried and visualized on a phosphorimager (Molecular Dynamics model 445 S1). Data were plotted as graphs of the activity of the bands at the pause site as a proportion of the total activity in the lane to show both concentration and temperature dependence (Figure 7).

Sequence of the template, PQ74:

5′-TCCAACTATGTATAC$(TTGGGG)_4$TTAGCGGCACGCAATTGCTATAGTG AGTCGTATTA-3′

Sequence of the primer:

5′-TAATACGACTCACTATAG-3′

Telomerase Assay

The assay was performed using 5′-end-biotinylated $d(TTAGGG)_3$ as a telomere as described previously.[11] Telomerase reaction mixtures were protected from light exposure during the reactions by using brown-colored tubes to avoid degradation of reaction components by TMPyP4. In brief, reaction mixtures (20 μl) containing 4 μl of cell lysate, 50 mM Tris-OAc (pH 8.5), 50 mM K-OAc, 1 mM $MgCl_2$, 5 mM β-mercaptoethanol, 1 mM spermidine, 1 μM telomere primer, 1.5 μM [α-^{32}P]-dGTP (800 Ci/mmol), 2 mM dATP, and 2 mM dTTP were incubated at 37 °C for 1 hr. Reactions were terminated by adding 20 μl of Streptavidin-coated Dynabeads suspension containing 10 mM Tris-HCl (pH 7.5) and 2 M NaCl. Streptavidin-coated Dynabeads bind selectively to the desired target (5′-biotinylated DNA), forming a magnetic bead-target complex. This complex was separated from the suspension using a magnet (Dynal MPC) and washed several times with washing buffer (1M NaCl) to eliminate [α-^{32}P]-dGTP background. Telomerase reaction products were separated from the magnetic beads by protein denaturation with 5.0 M

guanidine-HCl at 90 °C for 20 min. After ethanol precipitation, the reaction products were analyzed by 8% polyacrylamide gel electrophoresis. An additional experiment is shown in Figure 8 (left panel).

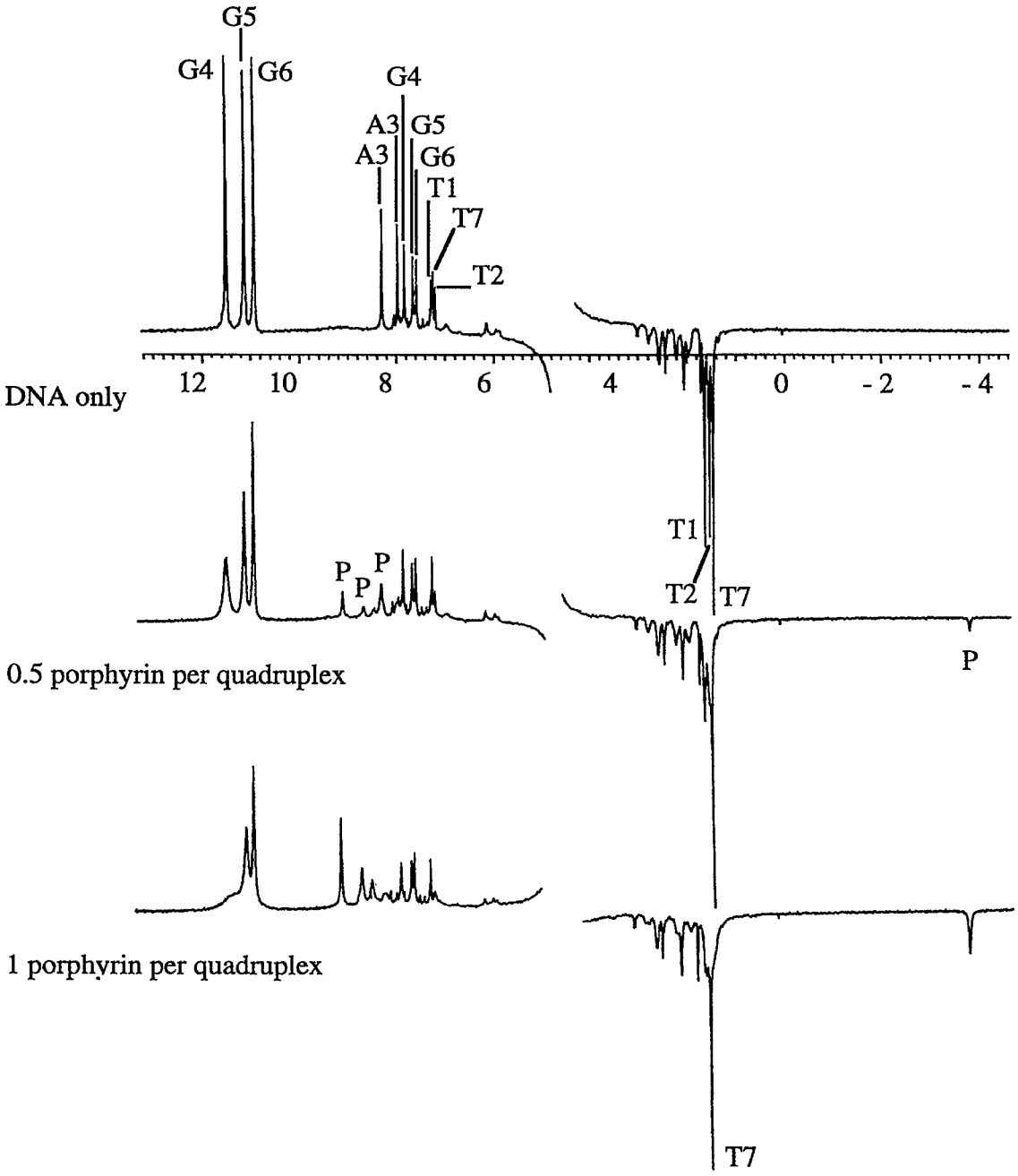

Figure 4. ^{1}H NMR Spectra of the titration of TMPyP4 into the d(TTAGGGT) quadruplex; the porphyrin resonances are marked P.

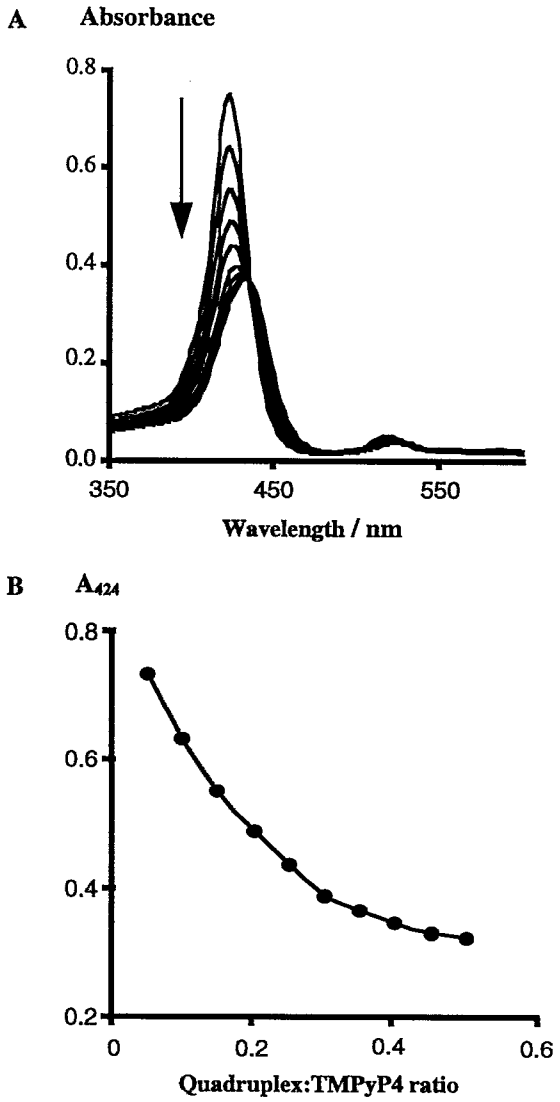

Figure 5.
(A) UV Titration of $d(AG_3[T_2AG_3]_3)$ into a solution of TMPyP4, monitoring the porphyrin soret band; (B) plot of the change in the porphyrin absorbance against quadruplex:TMPyP4 ratio showing that the hypochromic effect is effectively complete at a 1:2 ratio.

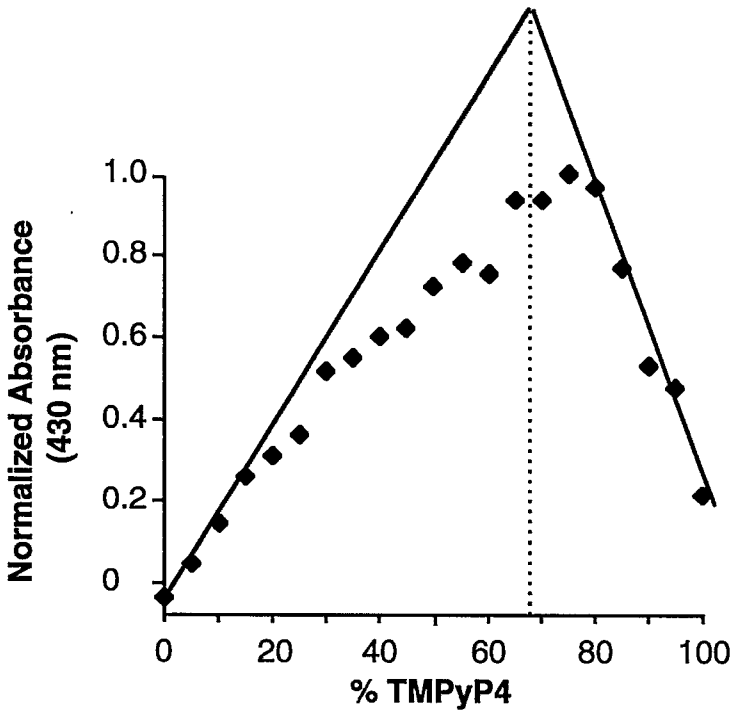

Figure 6.
Job plot for the complexation of TMPyP4 with $d(AG_3[T_2AG_3]_3)$. Extrapolation of the linear parts of the plot confirmed a 2:1 TMPyP4:quadruplex complex.

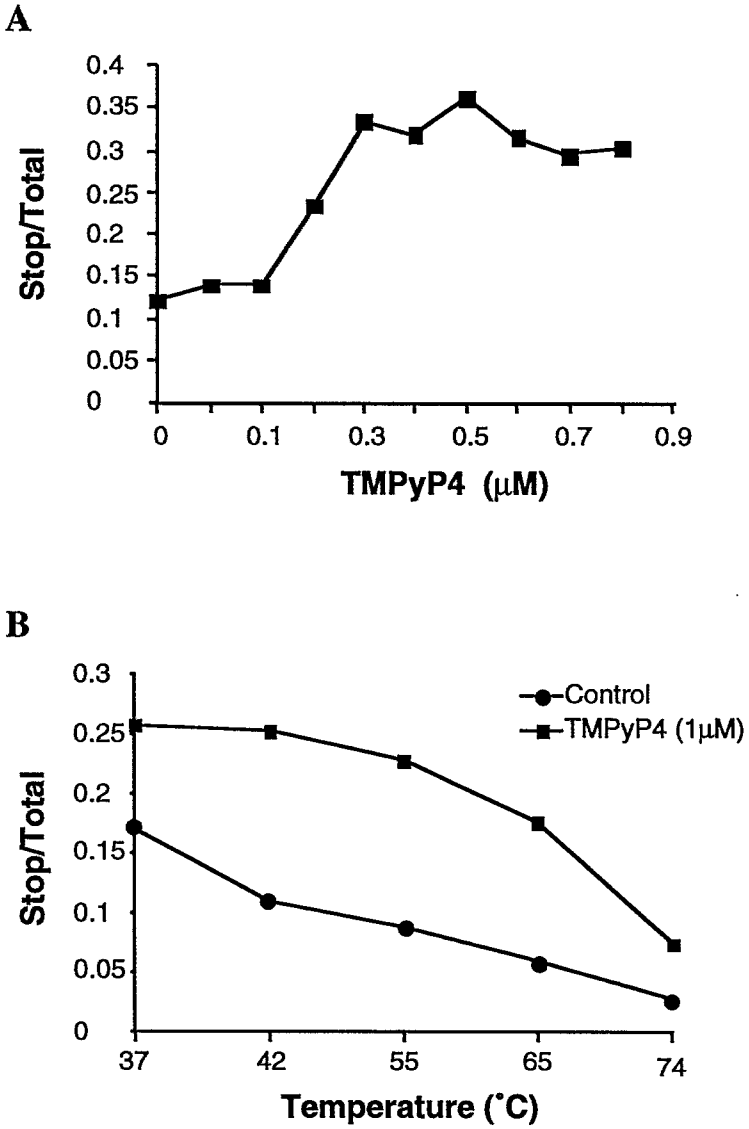

Figure 7.
Quantitation of the data of Figure 2. Radioactivity associated with the arrest site as a proportion of the total activity in the lane showing (A) Concentration dependence, and (B) temperature dependence of the block to Taq DNA polymerase. Data are the mean of 3 repetitions.

Fig. 8