Supporting Experimental Methods

UV Melting. UV melting was performed on a Varian Cary 1E UV/Vis spectrophotometer fitted with a Peltier temperature controller. The absorbance at 260 nm (duplex) or 295 nm (quadruplex) (1) was monitored as a function of temperature while ramping between 0 or 10°C and 90°C at a rate of 0.5°C·min^{-1}. The samples contained 2 μM **I** or **I**·**II** or 3 μM **IV**, 10 mM Tris·HCl (pH 7.4), and one of either 10 mM NaCl, 100 mM NaCl, or 100 mM KCl. Both heating and cooling curves were collected. The data were subjected to van't Hoff analyses to give the melting temperature, T_m, as the temperature at which the structure was 50% formed, i.e., when $\Delta G = \Delta H - T\Delta S = 0$.

CD Spectroscopy. Circular dichroism spectra of 2 μM **I**·**II** or **V**·**II** were taken on a Jasco J-810 spectropolarimeter in the presence of 10 mM Tris·HCl (pH 7.4) and either 100 mM NaCl or 100 mM KCl. Spectra of both buffers alone were also taken to correct for background readings. Spectra were taken as the average of 10 scans between 220 and 320 nm.

Fluorescence Anisotropy. Fluorescence anisotropies of TMR and Cy5 in **I**·**II** were measured on an Aminco-Bowman Series 2 Luminescence Spectrometer. The values are 0.22 ± 0.01 for both dyes in 10 mM Tris·HCl/100 mM NaCl and 10 mM Tris·HCl/100 mM KCl.

Determination of Experimental Error and Shot-Noise-Induced Peak Width. All errors quoted in the main text were calculated by standard statistical methods and are the standard deviation of the respective measurements. The upper limits of the shot-noise-induced FRET distribution width, ΔE, were estimated by the following formula (2):

$$\Delta E = \sqrt{E_m(1 - E_m)/T}$$

where E_m is the mean FRET value and T is the threshold value for the sum of the donor and acceptor fluorescence counts.

Removal of the "Zero" Peaks in the Single-Molecule FRET Histograms. The shape of the "zero" peak was recorded by measuring the single-molecule FRET histogram of a fully hybridized quadruplex/duplex system. This peak was fitted to two Gaussian functions. These functions were then used to fit the "zero" peak, and this was subtracted from the FRET histograms. For kinetic experiments, the "zero" peak at $t = 0$ was subtracted from the histograms recorded at different times.

Molecular Modeling. Molecular modeling of **I**·**II** was carried out using MACROMODEL 5.5 (3). All modeling of DNA was performed using the AMBER* forcefield and GB/SA implicit solvation. Structures of the parallel and antiparallel intramolecular quadruplexes were obtained from the Protein Data Bank, model 1kf1 and an arbitrarily chosen model from 143d, respectively. The 5'-adenines of these sequences were removed because they are not present in **I**. Model 1kf1 was modified by the removal of water molecules and potassium ions and the addition of hydrogen atoms. Models of **I**·**II** in both conformations were made by coupling the resulting structures to a model of the duplex region, created within MACROMODEL, by a DNA phosphodiester backbone linkage. Terminal phosphates were changed to hydroxyl groups.

Energy minimizations were performed by using the Polak-Ribiere conjugate gradient (PRCG) algorithm to an energy gradient of 0.01 $kJ \cdot mol^{-1} \cdot Å^{-1}$. The duplexes were truncated 8 bp from the quadruplexes to increase the speed of subsequent computations. Conformational searches were performed on the minimized structures by using the Systematic Unbounded Multiple Minimum (SUMM) algorithm to a maximum resolution of 30°. The search was carried out over three of the torsion angles of the linker region, i.e., G21ζ, A22α, and A22β of **I**, similar to the method used by Chaires and coworkers (4). For technical reasons, it was necessary to minimize the structures produced by the search. This minimization was kept as short as possible to reduce the computational time needed for this study. The shortened duplexes of the lowest-energy models of both systems were replaced by the full-length 35-bp duplex, and the resulting structures were minimized as above. Models of the dyes, created within MACROMODEL, were attached to

the DNA to give complete models of **I**·**II**. No attempt was made to optimize the positions of the dyes relative to the DNA.

Estimate of Δ*l*. Because the donor fluorophore is situated on the duplex region 8 bp from the quadruplex, a structural transition from antiparallel to parallel conformations is not likely to affect the conformation of its linker with respect to the duplex. However, because the vector between the TMR and Cy5 attachment sites differs between the structures, it could still contribute to the difference between the linker contributions to the interfluorophore distance in the two models. The distance between the TMR, placed in an arbitrary location relative to its attachment point, and the Cy5 attachment point can be determined by using vector arithmetic. If it is assumed that the TMR vector is the same with respect to the duplex in both structures, then it is possible to work out the contribution of the TMR linker, l_{TMR} to l, for each structure and, hence, the contribution of the TMR linker to Δl. The SOLVER module of Microsoft EXCEL was used to find the maximum contribution of the TMR linker to Δl by varying the TMR position under the restraint that it was, at most, 1.7 nm from its attachment point. Because the position of the Cy5 is likely to vary between the structures, its contribution to Δl was taken to be twice its linker length, i.e., 1.8 nm.

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