Ultrafast energy transfer within pyropheophorbide-a tethered to self-assembling DNA quadruplex

Jianfeng Cai[1], *Dariusz M. Niedzwiedzki*[2], *Harry A. Frank**[2], *and Andrew D. Hamilton**[1]

[1]Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 06511

[2]Department of Chemistry, University of Connecticut, Storrs, CT 06269

andrew.hamilton@yale.edu and harry.frank@uconn.edu

Experimental section

Materials and Methods

Pyropheophorbide-a was purchased from Frontier Scientific, Inc. without further purification. The core oligodeoxynucleotide (ODN) sequences were synthesized by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University using automated solid phase synthesis. The modified phosphoramidite 5'-amino-modifier C6 was obtained from Glen Research. The oligonucleotides were first subject to gel filtration using Microspin G-25 columns (GE health care), then purified by reverse-phase HPLC (Varian Prostar) equipped with a timberline TL-105 column heater. The concentrations of the purified oligonucleotides were assessed based on their absorption at 260 nm using an Agilent A453 UV-vis spectrometer. The molecular weights of the purified ODNs were determined by Matrix-Assistant Laser Desorption Ionization-Time of Flight (MALDI-TOF) using an Applied Biosystems Voyager-DE PRO workstation. The formation of DNA quadruplexes was assessed by circular dichroism (CD) spectra.

Standard preparation of oligonucleotides

Cartridges containing resin tethered with the amino modified ODN were obtained for the purpose of DNA isolation. Activated pyropheophorbide solution was prepared by mixing 4 mg of pyropheophorbide-a, 4 mg of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (Pybop), 1 mL of anhydrous dimethylforamide (DMF), and 50 μl N,N'-Diisoproplyethylamine (DIPEA) in a vial and stirring in the dark for 2 minutes. The solution was then transferred to syringes and filtered through the resin cartridges 5 times, followed by agitation for 1 hour. This procedure was repeated in quintuplicate, after which time the cartridges were placed on a shaker to stir overnight. The following morning the solution was removed and the cartridge was washed sequentially with 2 mL DMF and 2 mL acetonitrile, both in triplicate. The resin was dried under argon gas for 1 hour. DNA was then cleaved from the solid support by flushing the cartridge with 30% NH_4OH for 2 hours at room temperature, and subjecting the cartridge to global deprotection by soaking with 3 ml of 30% NH_4OH at 55°C for 16 hours.

The ODNs on a solid support were tethered with a C6 amino modifier, which then coupled with pyropheophorbide-a by Pybop and DIPEA in DMF for 12 h. The pyropheophorbide-a tethered ODNs were incubated in concentrated aqueous ammonia at room temperature to cleave them from the resin and then subjected to global deprotection at 55ºC for 16 h. The ODN conjugates were purified by high performance liquid chromatography (HPLC) at 65℃and confirmed by MALDI-TOF mass spectrometry using a 9:1:1 mixture of 2,4,6-trihydroxyacetopheone (THAP) (10 mg/mL in 50% CH_3CN/water), ammonium citrate (50 mg/ml in water), and oligonucleotide solution, respectively. (Figure S1). Functionalized parallel quadruplex (**1X**) was prepared in 10 mM Tris-HCl, 80 mM KCl, pH 7.5 buffer and incubated for 48 hr.{Jayawickramarajah, 2007 #2}

Oligonucleotide **1X**. MALDI-TOF Calculated: 4128.8, found: 4125.0

Oligonucleotide **2X**. MALDI-TOF Calculated: 4290.06, found: 4286.3

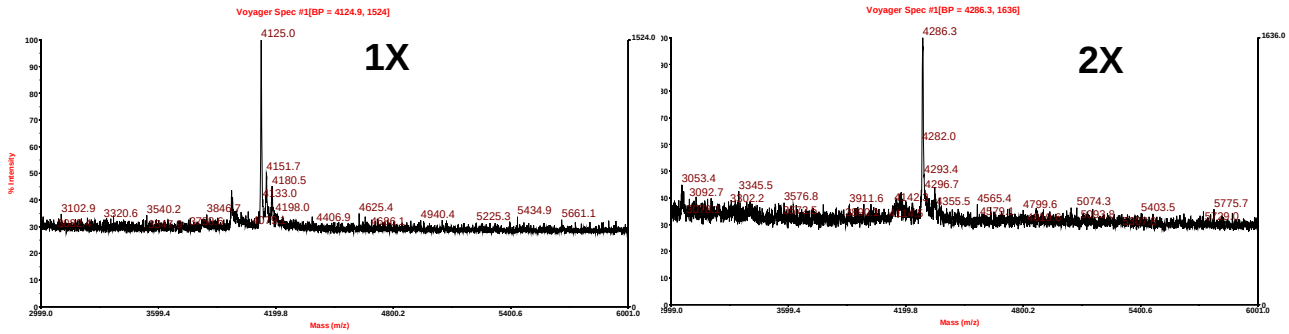


Figure S1. MALDI-TOF of **1X** and **2X.**

Self-Assembly of quadruplex DNA

The purified aliquot of **1X** was diluted into desired concentration by using 10 mM Tris-HCl, 80 mM KCl buffer with pH 7.5 in the eppendorf tube. Then the tube was tightly capped and sealed with parafilm, and heated at 95 ℃ for 15 min, and then slowly cooled to room temperature. Finally the solution was incubated at 4 ℃ for 48 h. The formation of quadruplexes was assessed by CD spectra.

Time-Correlated Single-Photon Counting Fluorescence Spectroscopy (TCSPC)

Fluorescence lifetime measurements were done using a TCSPC system of a Jobin-Yvon Horiba Fluorolog 3 equipped with a Single Photon Counting Controller FluoroHub 2.0 (J-Y Horiba), a Hamamatsu R928P photomultiplier tube detector, and a pulsed NanoLed-677L diode (as excitation light source), with excitation at 665 nm and a pulse duration less than 200 ps. The sample emission was monitored at 685 nm. Fitting of datasets was done using Data Analysis Software DAS v6.4 (JY Horiba).

Transient Absorption Spectroscopy

The ultrafast transient absorption system has been described in detail previously [1] and is based on an amplified Ti:Sapphire laser system (Spectra-Physics) operating at 1 kHz repetition rate. Pump pulses were obtained from an optical parametric amplifier (Spectra-Physics) with a pulse duration of ~120 fs, and the probe laser pulses were derived from a white light continuum, generated by a nonlinear crystal provided by Ultrafast Systems LLC. The pump and probe were overlapped at the sample at the magic-

angle (54.7°) polarization. A polarizer was placed in front of the spectrometer to minimize the scattered signal from the pump beam. For detection an Ocean Optics S2000 PCI charge-coupled detector was used.

The samples were adjusted to an optical density of 0.8 measured in a 2 mm path length cuvette at the excitation wavelength of 677 nm. The energy of the pump pulse was 1 μJ, corresponding to a photon density of 3.1×10^{14} photons·cm^{-2} for a spot size of the pump pulse of ~1.2 mm diameter. The samples were mixed continuously using a magnetic micro-stirrer to avoid photo-degradation. Also, sample integrity was confirmed by taking steady-state absorption before and after every experiment. Surface Explorer (v.1.0.6) (Ultrafast Systems LCC) software was used to correct for dispersion in the transient absorption spectra and also to determine the principle number of kinetic components by singular value decomposition (SVD). ASUFit 3.0 software provided by Dr. Evaldas Katilius at Arizona State University was used for global fitting analysis using sequential decay path model (EADS).

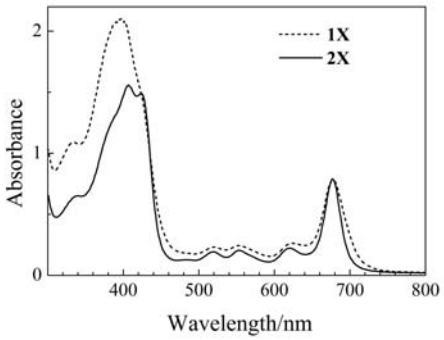


Figure S2. Steady-state absorption spectra of ODNs. **1X**, DNA quadruplex with 5' ends tethered with pyropheophorbides and **2X**, DNA single strand-pyropheophorbide adducts. The measurements were done in 80 mM KCl, 10 mM Tris-HCl, pH 7.5 buffer at room temperaure.

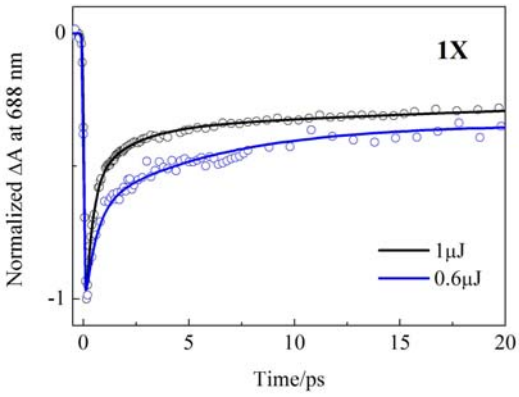


Figure S3. Laser power dependence of singlet-singlet annihilation of **1X**. The kinetics were taken at 688 nm and were normalized in amplitude for comparison. Solid lines represent the best fits obtained from global fitting analysis of the datasets.

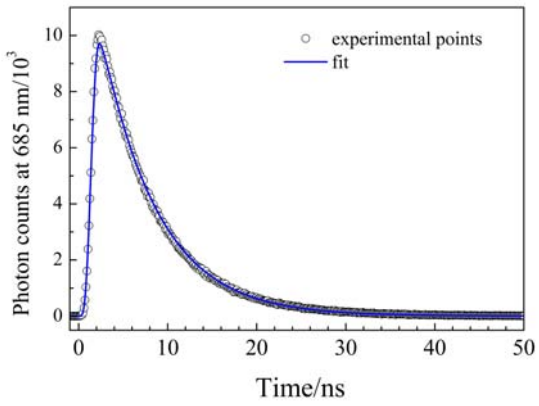


Figure S4. Fluorescence decay kinetics of **2X** measured at 685 nm (circles) with monoexponential fit (solid blue lines).

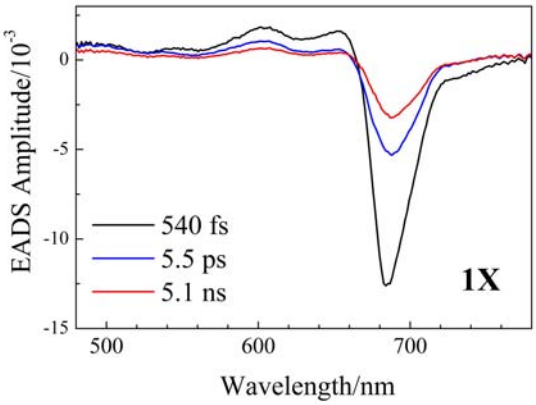


Figure S5. Global fitting of the transient absorption datasets of **1X** obtained using a sequential decay pathway of populated excited states. The line shapes obtained in this manner are termed evolution associated difference spectra (EADS). The results give an indication of the number of decay components as well as a reasonable approximation to the spectral features of the kinetic components. Three kinetic components were found to give a satisfactory fit in this case.

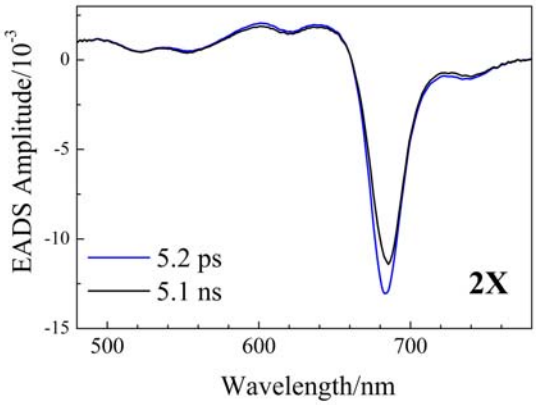


Figure S6. Global fitting results (EADS) of the transient absorption datasets of **2X**. Two kinetic components wre found to give a satisfactory fit.

Förster Energy Transfer Calculations

Pyropheophorbide-a ring was built in HyperChem v. 5.11 (Hypercube) and optimized using semi empirical AM1 method. For simplicity the pyropheophorbide-a ring was approximated by a 14 Å square profile (Figure S7).

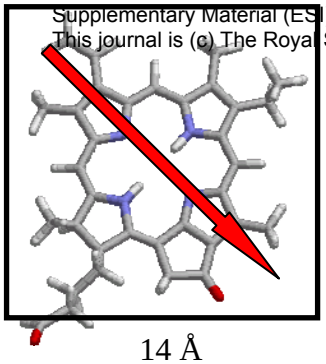


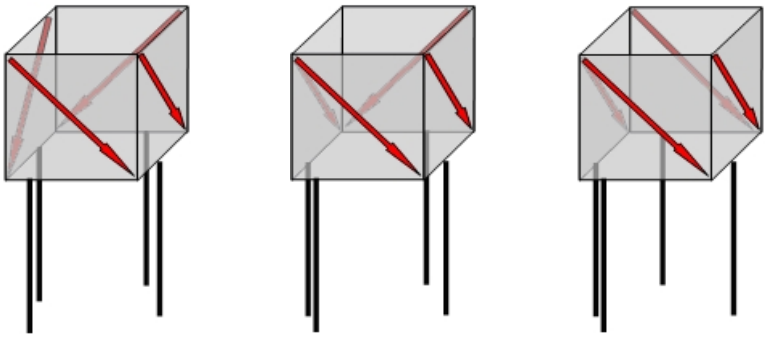
Figure S7. Simplified representation of the Pyropheophorbide-a ring as a square box with a 14 Å side dimensions. The red arrow represents the Qy transition dipole moment along the Y internal molecular axis.

For the Förster energy transfer calculations, three hypothetical tetrameric structures were built assuming that the tetramer formed idealized cubic structure (Figure S8). The microscopic rates of through-space energy transfer were then calculated on the basis of.

$$k_{TS} = 8.785 \times 10^{-11} \left(\frac{\kappa^2 \Phi_F}{n^4 R^6 \tau} \right) \int_{\lambda} F_D \lambda^4 \varepsilon_A d\lambda$$

where κ is the orientation factor, and constant values are assumed for the index of refraction, n = 1.33; fluorescence quantum yield, $\Phi_F = 0.32$ [2]; and fluorescence lifetime, $\tau_F = 5.1$ ns.

Figure S8. Three hypothetical structures of **1X** used for modeling the microscopic energy transfer rate constants.



1XA **1XB** **1XC**

Table S1. Förster Energy Transfer Rate Constants (k_{TS}), orientation factors (κ), distances (R), quantum fluorescence yields (Φ_F), and fluorescence lifetimes (τ_F).

	κ	R(Å)	Φ_F	τ_F (ns)	k_{TS} (s^{-1})
1XA neighbor-neighbor	1.25	9.9	0.32	5.1	11.8×10^{12}
neighbor-next neighbor	0	14.0	0.32	5.1	0
1XB neighbor-neighbor	1.25	9.9	0.32	5.1	11.8×10^{12}
	-0.25	9.9	0.32	5.1	47×10^{12}
neighbor-next neighbor	0	14.0	0.32	5.1	0
	1.00	14.0	0.32	5.1	94×10^{12}
1XC neighbor-neighbor	-0.25	9.9	0.32	5.1	47×10^{12}
	1.25	9.9	0.32	5.1	11.8×10^{12}
neighbor-next neighbor	1.00	14.0	0.32	5.1	94×10^{12}

References

(1) R. P. Ilagan, R. L. Christensen, T. W. Chapp, G. N. Gibson, T. Pascher, T. Polivka, and H. A. Frank. *J. Phys. Chem. A.*, 2005, **109**, 3120-3127.

(2) J. M. Dixon. Ph.D. thesis, North Carolina State University, Raleigh, North Carolina, **2004**.