Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Ultrafast molecular rotor: An efficient sensor for premelting of natural DNA

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Experimental Procedure

Steady-state fluorescence measurements were carried out using a Hitachi spectro fluorometer (model F-4500). Spectrum of standard quinine sulfate solution was recorded and compared with the standard spectrum,[1] to get the wavelength dependent correction factors for the instrumental sensitivity. These correction factors were used to correct the observed fluorescence spectra for the sample. In spectrofluorometer, temperature of the sample was controlled through circulating water and temperature was measured using thermometer inside the optical cell.

Time-resolved fluorescence measurements were carried out using a diode-laser based time-correlated single photon counting (TCSPC) spectrometer from IBH (UK). Diode lasers of 408 nm and 375 nm wavelength (1 MHz) were used for the excitation of ThT and DAPI respectively. A 560 nm nano LED (1 MHz) was used for the excitation of EB. MCP-PMT detector (IBH) was used for the fluorescence decay measurements. The instrument response function (IRF) of the TCSPC setup was measured by collecting the scattered light from a TiO_2 suspension in water. The IRF thus measured were ~120 ps, ~170 ps and ~1.3 ns for 408 nm, 375 nm and 560 nm excitation sources respectively. The decays were analyzed using IBH DAS-6 decay analysis software. The temperature of the solution was varied with the help of a cold finger arrangement and the temperature was controlled using a microprocessor-based temperature controller (model DS from IBH). For lifetime measurements, fluorescence decays were recorded at the magic angle (54.7°) with respect to the vertically polarized excitation light.

Thioflavin-T, (ThT) and Tris salt were purchased from Sigma. ThT was re-crystallized twice from methanol. The purity of the re-crystallized ThT was checked through NMR spectra. DAPI and EB were purchased from Sigma and used as received. Calf thymus DNA (CT DNA) from Sigma was used as received after checking its purity through absorption measurement. The ratio of the absorbance of the DNA solution at 260 nm to that of 280 nm was found to be 1.8, which suggest the absence of any protein impurities.[2] Deionized water (Millipore Gradient A10 system, conductivity less than 0.1 $\mu s \cdot cm^{-1}$) was used for all solution preparation. A stock solution of DNA was prepared by dissolving an appropriate amount of solid DNA in Tris-HCl buffer solution (5 mM, pH = 7.4) and stored at 4°C for

more than 24 hrs with occasional gentle shaking to get a homogeneous solution. DNA concentrations were determined by using an extinction coefficient of 6600 $mol^{-1}cm^{-1}$ at 260 nm and expressed in terms of base molarity.[3] For all experiments concentrations of DNA and dyes were used are 1mM and 10μM respectively.

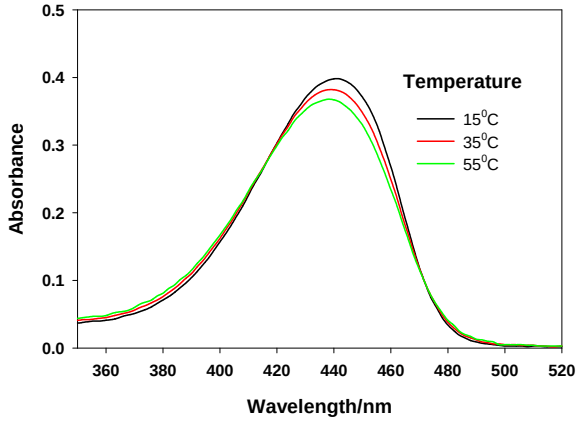

Fig. S1. Absorption spectra of ThT in DNA solution at different temperatures. It is evident that the changes in the DNA structure in the premelting region is reflected in the absorption spectra of ThT. However, the changes in the absorpbance of ThT in DNA solution due to change in the solution temperature from 15^0 to 55^0C is nominal (only 8%)

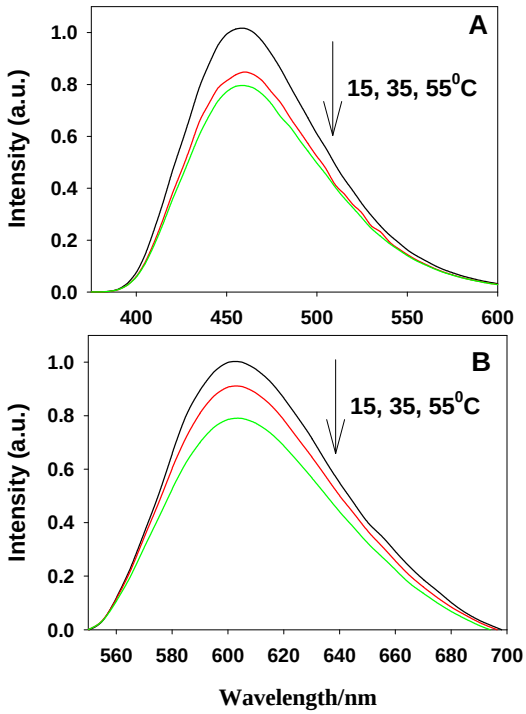

Fig. S2. Emission spectrum of (A) DAPI and (B) EB in DNA solution at different temperatures. In both cases the emission intensity decreases due to change in the solution temperature from 15^0 to 55^0C. The changes in the emission intensity for DAPI (20%) and EB (21%) is very less as compared to that for ThT (250%).

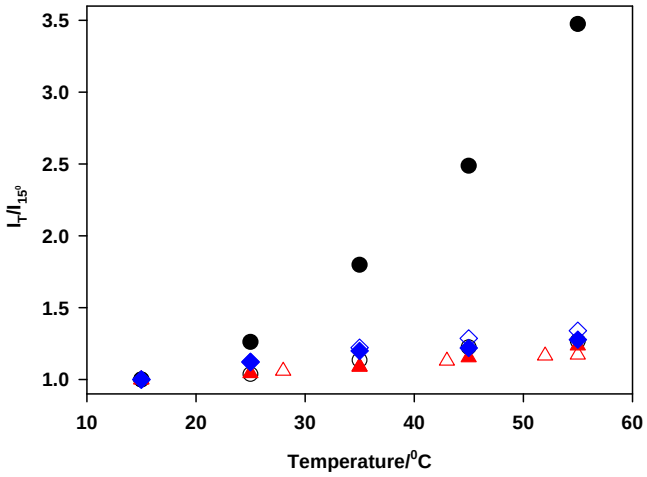

Fig. S3. Variation of the peak emission intensity (relative to that at 15^0C) with temperature for (●) ThT in DNA, (○) ThT in buffer, (▲) EB in DNA, (△) EB in buffer, (◆) DAPI in DNA and (◇) DAPI in buffer solution.

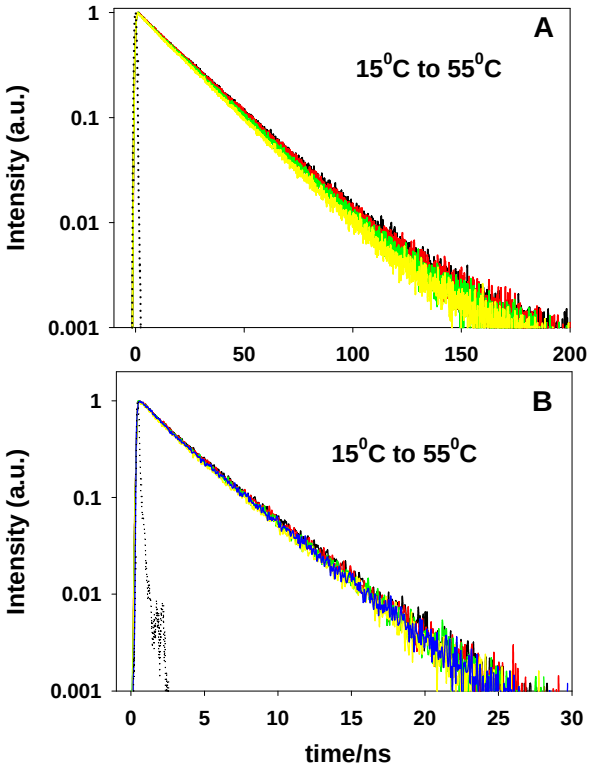

Fig. S4. Emission transient decay for (A) EB and (B) DAPI in DNA solution at different temperatures. Instrument response function (IRF) is shown by dotted curve.

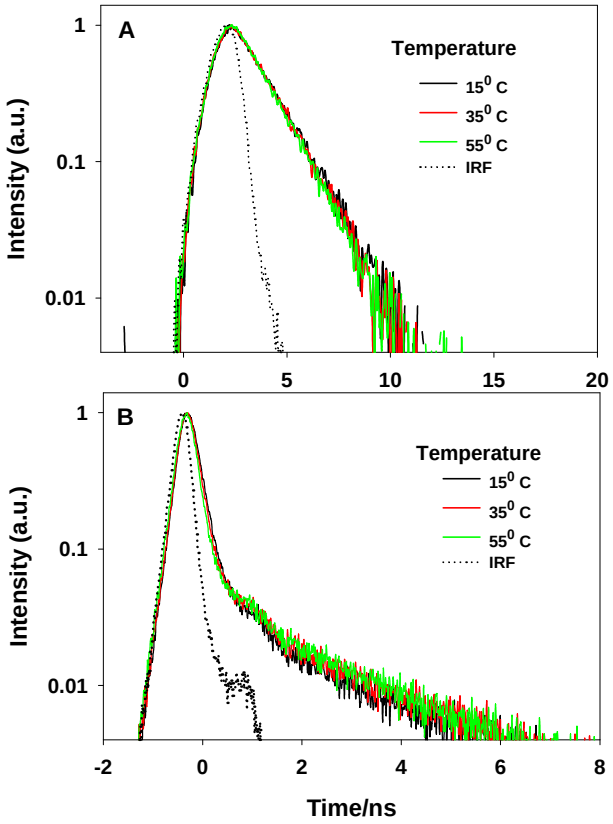

Fig. S5. Emission transient decay for (A) EB and (B) DAPI in buffer solution at different temperatures. Instrument response function (IRF) is shown by dotted curve.

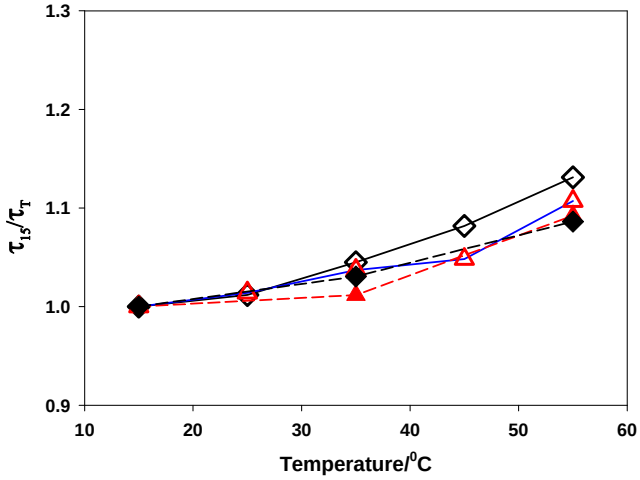

Fig. S6. Variation of the excited state lifetime (relative to that at 15^0C) with temperature for (◇) EB in DNA, (◆) EB in buffer, (△) DAPI in DNA and (▲) DAPI in buffer solution.

Reference:

1 R. A. Velapoldi and K. D. Mielenz, *Nat. Bur. Stand. (US) Sec. Publ.*, 1980, 260.
2 W. Saenger, 'Principles of nuclei structure', Spinger-Verlag, 1984.
3 J. K. Barton, J. M. Goldberg, C. V. Kumar, and N. J. Turro, *J. Am. Chem. Soc.* , 1986, **108**, 2081.