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White-Light-Emitting DNA (WED)

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White-light-emitting organic molecules have received great attention for potential applications in full-colour light emitting diodes (LEDs) and lighting purposes.[1] A common and efficient approach to obtain these organic materials is based on the partial energy transfer (δET) between donors and suitable acceptors. The acceptor is either doped in the donor scaffold or covalently attached to the donor and the system simultaneously emits over the whole visible range ($\lambda = 400$–750 nm). This approach has been successfully applied in polymers blended with luminescent dyes and other systems.[2,3] Moreover, white-light-emitting ensembles have been developed by making use of supramolecular recognition of specific non-covalent interactions encoded in the donor and acceptor molecules.[4,5] Notably, in all these cases, an efficient interaction and close proximity (Förster distance, <10 nm) of the donor and acceptor are essential for the fluorescence resonance energy transfer (FRET).

The characteristic structural features of DNA like its inherent double helical conformation and the typical stacking distance of ≈ 3.4 Å between the bases offers a unique structural scaffold for the helical organization of covalently attached chromophores with very efficient electronic interactions between them. This has been successfully exploited for the helical organization of various classes of π conjugated chromophores, which can provide an efficient medium for the migration of excitation energy for the development of photoactive and functional nanomaterials.[6–13]

Herein, we demonstrate how to use the DNA backbone for the combination of a donor and an acceptor in such a way that a white-light-emitting DNA is formed upon hybridization. We synthesized **DNA1** bearing the blue–green emitter, ethynyl pyrene (400–600 nm) and **DNA2** bearing the red emitter, ethynyl nile red (600–750 nm). Both chromophores were conjugated to the 5-position of 2′-deoxyuridine to maintain the canonical dU:A base pairing. In **DNA3-6** both chromophores were combined as an energy donor–acceptor couple and placed adjacent to each other in order to obtain an efficient electronic interaction between them (Figure 1). The phosphoramidite of 5-(1-pyrenylethynyl)-2′-deoxyuridine was commercially available. The corresponding phosphoramidite of 2′-deoxyuridine modified with ethynyl nile red was synthesized by palladium catalysed Sonogashira coupling of ethynyl nile red (which was synthesized according to the methodology in a recent literature report)[14] and 5′-*O*-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine followed by the phosphoramidite reaction under standard conditions.[15] Using both DNA building blocks, the modified oligonucleotides **DNA1-6** were synthesized by

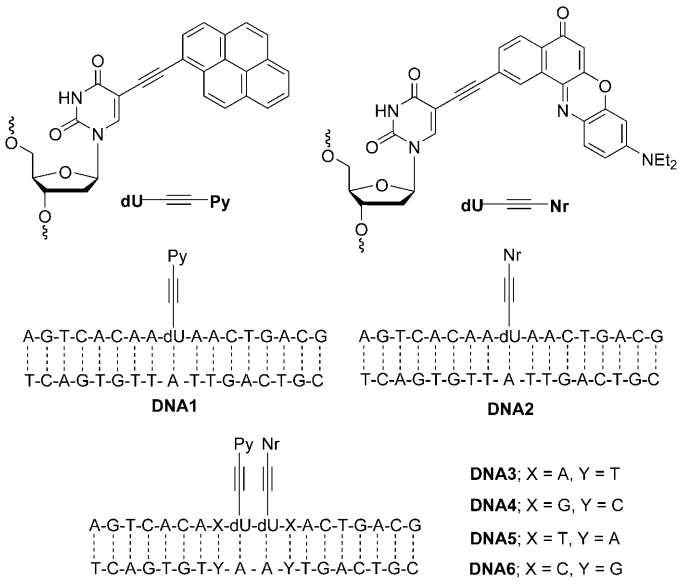

Figure 1. Structure of pyrene- and nile-red modified deoxyuridines and the DNA sequences used in this study.

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automated phosphoramidite chemistry without major changes to the standard procedures.[15]

The reference duplexes **DNA1** and **DNA2** showed the characteristic optical behaviour of the corresponding chromophores. **DNA1** exhibited the absorption and fluorescence maxima of pyrene at $\lambda = 401$ nm ($\varepsilon_{401nm} = 3.6 \times 10^4\,M^{-1}cm^{-1}$) and $\lambda = 440$ nm ($\phi_f = 0.15$), respectively, and **DNA2** showed an absorption maximum at $\lambda = 615$ nm ($\varepsilon_{615nm} = 2.5 \times 10^4\,M^{-1}cm^{-1}$) and emission maximum at $\lambda = 665$ nm ($\phi_f = 0.30$), respectively.[15] The calculated spectral overlap integral ($J(\lambda) = 1.5 \times 10^{15}\,M^{-1}cm^{-1}nm^4$) from the emission spectrum of **DNA1** and the absorption spectrum of **DNA2** reveals the possible non-radiative transfer of excitation energy. Moreover, the well separated absorption maxima and the high extinction coefficient of the acceptor were promising factors for the transfer of excitation energy. The extinction coefficient of ethynyl nile red ($\varepsilon_{380nm} = 1400\,M^{-1}cm^{-1}$) at the excitation wavelength of ethynyl pyrene is considerably low which diminishes the possibility of the direct excitation of the acceptor.

The absorption spectra of the double labelled **DNA3-6** showed the characteristic absorption of both ethynyl pyrene and ethynyl nile red and thus ruled out the possibility of any ground state interaction between them (single strand (ss) and double strand (ds)).[15] Accordingly, circular dichroism (CD) experiments showed no detectable signal in the absorption region of the chromophores, but confirmed a B-like helical conformation.[15] The thermal melting (T_m) studies revealed that the incorporation of a single chromophore destabilises the duplex by $-3.9\,°C$ for **DNA1** and $-3.6\,°C$ for **DNA2**. However, double incorporation (**DNA3-6**) regained the destabilization by the hydrophobic interactions between the chromophores.[15]

Excitation of ss-**DNA3** at $\lambda = 380$ nm resulted in 92% quenching of the pyrene fluorescence at $\lambda = 440$ nm with a concomitant formation of the nile red emission at 665 nm. As expected, a FRET occurs from pyrene to nile red in a non-radiative manner with a rate constant of $5.24 \times 10^9\,s^{-1}$.[15] Very interestingly, the corresponding ds-**DNA3** exhibited a decrease in the FRET efficiency (83%) since only a partial energy transfer ($k_{ET} = 1.69 \times 10^9\,s^{-1}$) occurs. The combined effects, an increase at $\lambda = 440$ nm and a decrease at $\lambda = 665$ nm (compared to the corresponding ss) yield a white emission, owing to the almost equal intensity for the blue–green and red emitting peaks ($I_{440}/I_{665} = 0.96$) (Figure 2a–c). A schematic representation of this FRET controlled emission changes is illustrated in Figure 2d. Even though the difference in the FRET is only $\approx 10\%$, the significant drop of the red emission intensity in the duplex is probably owed to the large molar extinction coefficient and the high fluorescence quantum yield of ethynyl nile red. The efficiency of dipole–dipole coupled Förster resonance energy transfer is highly dependent on the relative orientation of the dipole moments of the donor and acceptor.[16] The observed difference of the FRET efficiencies in the ss vs ds in the present system is likely owed to the change in the relative orientation of the chromophore dipole moments upon duplex formation (helically twisted conformation) which may prohibit an efficient energy transfer in the case of ds-**DNA3**.

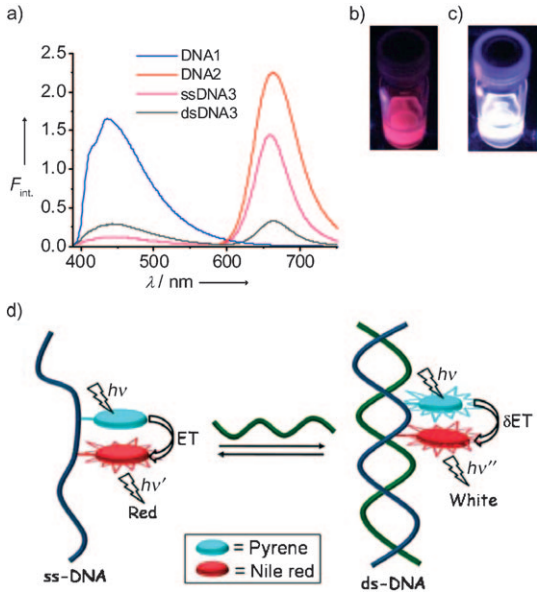

Figure 2. a) Fluorescence spectra of **DNA1** (blue), **DNA2** (orange), ss-**DNA3** (red) and ds-**DNA3** (grey) ($c = 2.5 \times 10^{-6}$ M in Na–P_i buffer, 250 mM NaCl, pH 7). $\lambda_{exc} = 380$ nm for **DNA1**and **DNA3** and $\lambda_{exc} = 580$ nm for **DNA2**. Photographs of the solution of **DNA3** b) ss and c) ds under illumination with $\lambda = 380$ nm UV light. d) A schematic representation illustrating the hydridization induced emission colour change.

As the neighbour bases adjacent to the chromophores affect significantly their photoluminescence behaviour, owing to various side effects, such as charge transfer, exciplex formation etc., we synthesized **DNA4-6** to investigate the effect of the base pairs adjacent to the chromophores on the hybridization induced white-light emission. As expected, emission spectra of all three single strands (ss-**DNA4-6**) exhibited a major peak at $\lambda = 665$ nm when excited at $\lambda = 380$ nm, owing to an efficient energy transfer from pyrene to nile red similar to ss-**DNA3** (Figure 3a). Remarkably, the fluorescence spectra of all three corresponding duplexes both blue–green and red emitting peaks of almost equal intensity ($I_{440}/I_{665} = 0.6$, 1.0, and 1.1 for **DNA4**, **DNA5** and **DNA6**, respectively) and hence yielded white emission (Figure 3b). Only when guanines are located adjacent to the chromophores (**DNA4**) a low value of I_{440}/I_{665} (0.6) was observed even though the emitting light is still nearly white in colour. Time resolved spectroscopic studies also support non-radiative FRET from pyrene to nile red. The fluorescence decay profile of **DNA3** ($\lambda_{exc} = 280$ nm) when probed at $\lambda = 440$ nm showed a biexponential decay with a major component of ≈ 3 ns (91%). On the other hand, ss-**DNA3** exhibited a triexponential decay with two major components having time constants, $\tau_1 \approx 1$ ns (65%), and $\tau_2 \approx 3$ ns (25%). This faster decay (τ_1) of the donor in the presence of the acceptor dye indicates the nonradiative energy transfer from pyrene to nile red.[4a] Interestingly, ds-**DNA3** also exhibited a triexponential decay with almost same time constants but with different amplitudes $\tau_1 \approx 1$ ns (52%) and $\tau_2 \approx 3$ ns

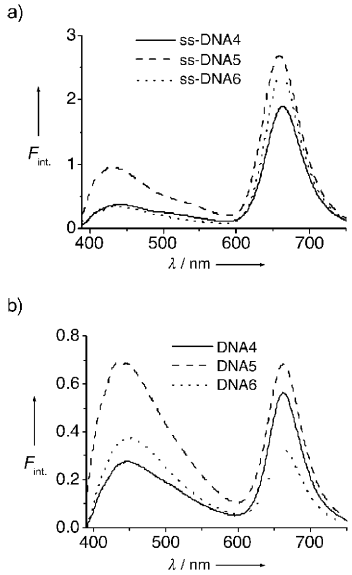

Figure 3. Fluorescence spectra of **DNA4-6** a) ss and b) ds ($c = 2.5 \times 10^{-6}$ M, in Na–P_i buffer, 250 mM NaCl, pH 7, $\lambda_{exc} = 380$ nm).

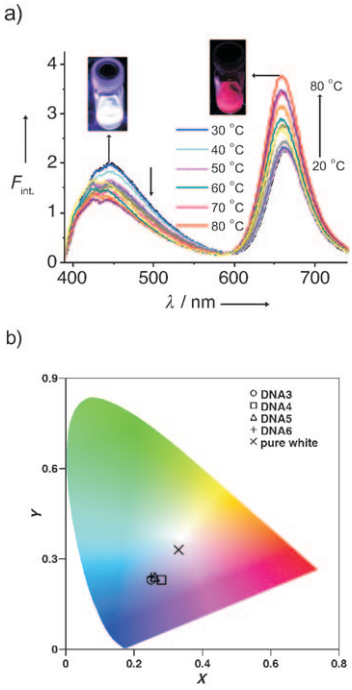

Figure 4. a) Temperature-dependent fluorescence spectra of ds-**DNA3** recorded in an interval of 5 °C ($c = 2.5 \times 10^{-6}$ M, in Na-P_i buffer, 250 mM NaCl, pH 7, $\lambda_{exc} = 380$ nm) and b) position of the fluorescent spectra of ds-**DNA3-6** in the CIE colour diagram.

(26 %). This decreased amplitude of τ_1 can be attributed to the decreased efficiency of the FRET in the duplex which stands in agreement with the observed steady state spectra. It should be noted that in all cases we observed a long lived species with different life times and its nature is not clear at present.

The major attractive feature of WED is the dependency of the energy transfer efficiency with the association/dissociation of the duplex which can be controlled by temperature. Accordingly, the intensity of the fluorescence peak of ds-**DNA3** at $\lambda = 665$ nm was found to increase, whereas the peak at $\lambda = 440$ nm decreased at high temperature when going from 25 °C to 75 °C. Concomitantly, the emission colour of the DNA changes from white to red (Figure 4a). Moreover, this colour change is completely reversible, as the dissociated duplex at high temperatures can be reassembled by cooling down the sample. Finally, the emitted white light is assigned in photometric terms, as standardized by the Commission Internationale de I′Eclairege (CIE). The CIE coordinates of ds-**DNA3-6** when their respective photoluminescence spectra were converted into chromaticity coordinates on a CIE 1931 diagram are (0.25, 0.23), (0.28, 0.23), (0.26, 0.24) and (0.25, 0.24) for **DNA3**, **DNA4**, **DNA5** and **DNA6**, respectively (Figure 4b). The CIE coordinates for absolute white light are (0.33, 0.33). It should be noted that WED deviate slightly from the CIE coordinates for an absolute white light, owing to the insufficient green emitting region and experiments are progressing in our laboratory in this direction.

In conclusion, we have demonstrated, for the first time, the potentiality of DNA scaffold to assemble a suitable donor–acceptor couple on DNA backbone towards the development of white-light-emitting systems. Moreover, the temperature controlled self-assembled association and dissociation of two oligonucleotides into the ds and back into the ss yields fully reversible emission colour change by modulating the FRET efficiencies. Hence, this new example of a DNA-based dynamic white-light-emitting assembly could be an entry to the class of white-light-emitting materials. Moreover, such a system will be a promising candidate as a nucleic acid probe to image DNA hybridisation by distinct emission colour changes upon duplex formation.

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Keywords: DNA · fluorescent probes · FRET · multichromophores · white-light emission

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