DNA as a supramolecular scaffold for the helical arrangement of a stack of 1-ethynylpyrene chromophores†

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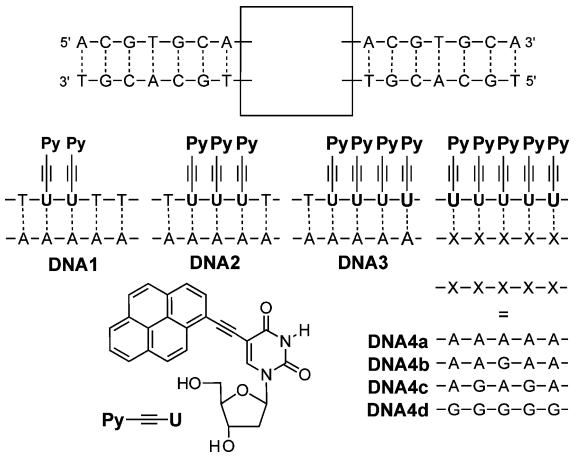
A highly organized helical π-stacked arrangement of 1-ethynylpyrene moieties along the major groove of duplex DNA can only be achieved if more than three chromophores have been synthetically incorporated adjacent to each other.

The generation of functionalized supramolecular nucleic acid architectures has important applications in nanobiotechnology.[1] Currently, the modification of nucleic acids by dual fluorescent labelling is routinely applied for molecular diagnostics.[2] DNA was also used as a template for the helical assembly of non-covalently bound chromophores.[3] The double-helical structure of DNA enables the placement of several adjacent chromophores in such a way that they photophysically interact with each other.[4] Pyrenes have been successfully used as optical probes for nucleic acids[5] and a helical π-array of four pyrenes along duplex RNA was published by Yamana and Nakamura *et al.*[6] Moreover, we showed that a π-array of five pyrene chromophores exhibits strong fluorescence enhancement that is sensitive to DNA base mismatches.[7]

Recently, we[8] and others[9] introduced 1-ethynylpyrene as a versatile fluorescent probe for DNA. Dual labelling of DNA by 5-(1-ethynylpyrenyl)-2′-deoxyuridine allows to study the dynamics of DNA chromophore interactions.[10] Herein, we want to present the optical properties of DNA helices that have been functionalized by several adjacent Py–≡–dU units. Using published protocols,[8,9] we prepared a range of DNA duplexes (**DNA1**–**DNA4a**, Scheme 1) that bear 2–5 adjacent Py–≡–dU chromophores. Three derivatives of **DNA4a** were synthesized bearing one (**DNA4b**), two (**DNA4c**) or five (**DNA4d**) guanines instead of adenine as part of the counterstrand.

The UV/Vis spectra of **DNA1**–**DNA4a** (Fig. 1) exhibit two main bands (380 and 405 nm) in the pyrene absorption range. Both peaks are red-shifted by *ca.* 5 nm and are intensified compared to the absorption of single Py–≡–dU-labelled DNA (peaks at 375 and 400 nm) that have been described by us and other groups.[8,9] Remarkably, the absorption is increasing more significantly with the number of pyrenes than it would be expected based on linearity. These absorption properties indicate ground-state interactions between the Py–≡–dU chromophores which exist already in **DNA1** but get more intensive especially in **DNA3** and **DNA4a** bearing four and five Py–≡–dU units. This interpretation is supported by the temperature-dependent UV/Vis spectra which are representatively



Scheme 1 Sequences of Py–≡–dU-modified duplexes **DNA1**–**DNA4d**.

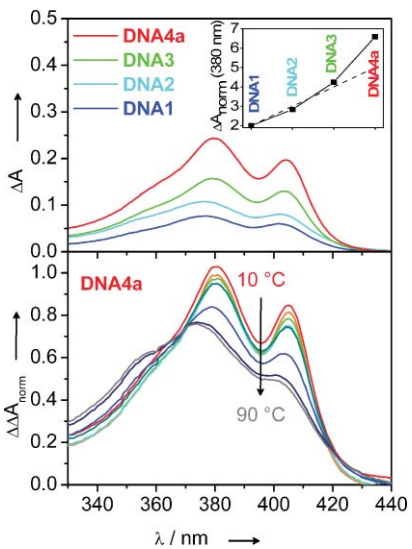


Fig. 1 Absorption spectra of **DNA1**–**DNA4a**, top, and temperature-dependent for **DNA4a**, bottom (2.5 μM in 10 mM Na–P_i buffer, 250 mM NaCl, pH 7). The inset shows the increasing absorption at 380 nm that is higher than linearity (dashed line).

shown for **DNA4a**. The red-shift, as well as the intensification as mentioned above, vanishes at a temperature of *ca.* 70 °C. The shape of the UV/Vis spectra of **DNA4a** above 70 °C look similar to the spectra of single Py–≡–dU-labelled DNA duplexes at r.t.[8,9] This temperature effect is also observed with **DNA3** and **DNA4b**–**DNA4d** (see the ESI). These results indicate the existence of a

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Table 1 Melting temperature (T_m) of **DNA1**–**DNA4d** (λ = 260 nm, 20–90 °C, 2.5 μM DNA in 10 mM Na–P_i buffer, 250 mM NaCl, pH 7)

Duplex	T_m (°C)	Duplex	T_m (°C)
DNA1	66	**DNA4a**	76
DNA2	65	**DNA4b**	72
DNA3	67	**DNA4c**	67
		DNA4d	71

π-stacked, ordered structure among the Py–≡–dU moieties in **DNA4a** that breaks down with the thermal denaturation of the DNA.

In order to evaluate the thermal melting behaviour of the Py–≡–dU-modified duplexes we measured the melting temperatures (T_m) at 260 nm (Table 1). **DNA4a** shows a remarkable stabilization compared to the others that is the result of the incorporation of five chromophores. One mismatch (**DNA4b**) or two mismatches (**DNA4c**) in this stack destabilize the chromophore arrangement in a similar way to melting calculations[10] with one and two GT mismatches in the corresponding unmodified duplexes. This is a remarkable result since it indicates that the base-pairing of Py–≡–dU with the correct counterbase is needed for the highly ordered arrangement of the chromophores. Interestingly, the complete change of the counterbases from adenines to guanines (**DNA4d**) allows the duplex to regain some stabilization.

The existence of ground-state interactions among the Py–≡–dU units should yield a detectable signal in CD spectroscopy (Fig. 2). In fact, the CD spectra of **DNA2** show a small and **DNA3** and **DNA4a** show a strong excitonic signal in the 1-ethynylpyrene absorption range between 330 and 430 nm. This CD signal supports clearly the interpretation of a helical arrangement of Py–≡–dU groups along the DNA axis. Remarkably, the absence of any significant CD signal in case of **DNA1** shows that at least three chromophores are needed as a critical amount for such a ordered structure. This result is supported by the absorption enhancement that has been observed for **DNA3** and **DNA4a**.

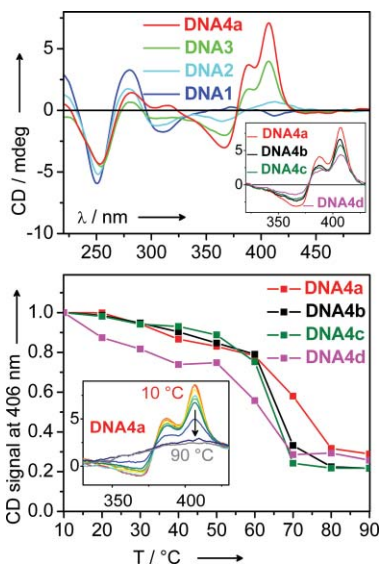

Fig. 2 CD spectra of **DNA1**–**DNA4d** (top: r.t., bottom: temperature-dependent) (2.5 μM in 10 mM Na–P_i buffer, 250 mM NaCl, pH 7).

Interestingly, as already indicated by the T_m values, the placement of base mismatches (**DNA4b**–**DNA4d**) decreases the CD signal maximum at 406 nm. Temperature-dependent CD spectroscopy (Fig. 2, bottom) shows the influence of base mismatches on the ordered π-stacked array of Py–≡–dU chromophores. The corresponding CD signal with a maximum at 406 nm exhibits clearly the DNA-typical melting behaviour. The breakdown of the DNA helix as the structural scaffold occurs in **DNA4b**–**DNA4d** at lower temperatures due to the conformational perturbation of the guanines as the "wrong" counterbases.

Finally the emission properties have been characterized (Fig. 3). As expected the emission intensity increases with the number of Py–≡–dU chromophores within the duplexes **DNA1**–**DNA4a**. The emission maxima vary between 485 and 494 nm and are all significantly red-shifted by at least 40 nm compared to single Py–≡–dU-labelled DNA (maximum at 445 nm).[8,9] This result again supports the idea of a strong ground-state interactions between the Py–≡–dU units meaning that in **DNA1**–**DNA4a**, excitation of ensembles of s stacked Py–≡–dU labels is preferred compared to the excitation of single unstacked Py–≡–dU moieties.

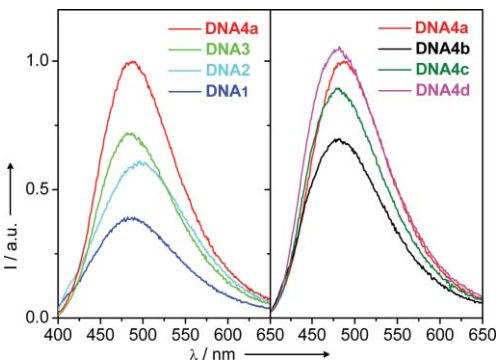

Fig. 3 Fluorescence spectra of **DNA1**–**DNA4d** (λ_{exc} = 375 nm, 2.5 μM in 10 mM Na–P_i buffer, 250 mM NaCl, pH 7).

The incorporation of one mismatch (**DNA4b**) strongly decreases the emission intensity as it interferes with the highly ordered structure of Py–≡–dU chromophores. Interestingly, the emission intensity increases again with a higher number of guanines in the counterstrand (**DNA4c** and **DNA4d**) that tracks with the observed T_m values. Obviously, in **DNA4d** the replacement of all five adenines to guanines results in an alternative conformation that allows a different Py–≡–dU interaction compared to that of **DNA4a** yielding slightly changed fluorescence properties.

In conclusion, it becomes clear that DNA duplexes are suitable supramolecular scaffolds for the helical arrangement of chromophores. At least three chromophores, and more importantly, an intact DNA helix environment are needed to obtain a highly ordered π-stacked array of chromophores. Especially the latter result supports the potential of this kind of DNA systems for applications in nanobiotechnology, such as fluorescent biosensors, and for the investigations of DNA dynamics.[11]

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