DNA Structures

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Duplex Stabilization and Energy Transfer in Zipper Porphyrin–DNA**

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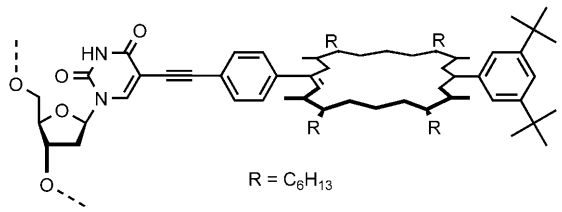
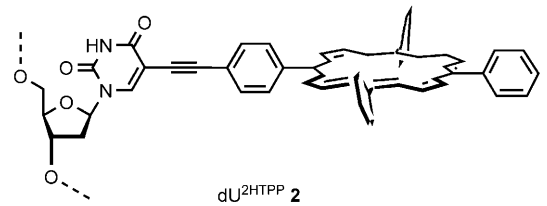
The use of functionalized DNA in the construction of new materials for potential nanotechnological applications is becoming more and more widespread.[1] In particular, the site-specific incorporation of fluorophores or metal complexes into DNA has led to the creation of supramolecular arrays with promising properties in optoelectronics. Both the interior[2] and exterior[3] of the DNA or RNA duplex are being used for attachment of modifications, and DNA proves to be a versatile supramolecular scaffold to create helical arrays of functional entities. Since our initial proposal to use DNA as a scaffold for porphyrin arrays[4] as compared to noncovalent assemblies,[5] we have studied both tetraphenylporphyrin (TPP) and diphenylporphyrin (DPP)-substituted DNA[6] and found significant differences in the thermal stability and the electronic properties of the multiporphyrin systems.[7,8] The attachment of substituents on one strand has an especially profound impact on the stability of the DNA duplex. In contrast to this one-strand modification, individual porphyrins,[9,10] metal-chelating ligands,[11] and pyrene–perylene systems[12] have been attached to both complementary strands, which leads to new supramolecular systems after hybridization. The design of longer zipper arrays based on an RNA or DNA scaffold, which have been reported by the groups of Wengel,[13] Leumann,[14] Häner,[15] and Wagenknecht,[16] is also very intriguing. Herein, we report that mixed porphyrin arrays can be created through a zipper-like arrangement by modifying both complementary strands, which can lead to a stabilization of the DNA duplex and resonance energy transfer between the chromophores after hybridization.

The two porphyrin-modified deoxyuridines, in which a diphenylporphyrin (dU^{2HDPP}, **1**) or a tetraphenylporphyrin (dU^{2HTPP}, **2**) are attached to the 5-position of the nucleobase, can be incorporated into DNA using standard solid-phase synthesis (Scheme 1). The destabilization of the DNA duplex in the one-strand-modified DNA is highly dependent on the nature, the number and the sequence of the porphyrin modification. In all cases we found a leveling of the destabilization effect at higher numbers (>4 modifications) of porphyrin groups in the DNA. TPP destabilized the DNA duplex by about −3 °C per porphyrin, and DPP by about −7 °C per porphyrin.



dU^{2HDPP} **1**



dU^{2HTPP} **2**

3a,b 5'-GAGTACGG**X**A**X**ACCGTACTC-3'
4a,b 5'-CATCGTAGTTTT**Y**A**Y**TTTTCCGTACTC-3'
5a,b 3'-GTAGCATCAAAAA**Y**AAAAAGGCATGAG-5'
6a,b 5'-CATCGTAG**Y**A**Y**A**Y**A**Y**A**Y**CCGTACTC-3'
7a,b 3'-GTAGCATCA**Y**A**Y**A**Y**A**Y**A**Y**AGGCATGAG-5'
8a,b 5'-CATCGTAGTA**X**A**X**A**X**A**X**ATCCGTACTC-3'

a unmodified strand; **X,Y**=T
b porphyrin-DNA; **X**=**1**, **Y**=**2**

Scheme 1. Structure and sequences of the porphyrin–DNA strands. Note, **8a** is identical to **6a**.

To test whether attachment of the porphyrins on complementary strands would alter the duplex stability, we synthesized the palindromic sequence **3b**, incorporating two modified groups **1** in the center, which will form a four-porphyrin array upon hybridization. The melting temperature of the unmodified self-complementary strand **3a**·**3a** is $T_m = 72$ °C, whereas for the porphyrin-substituted strand **3b**·**3b** it is $T_m = 61$ °C (Table 1). The ΔT_m of −10.4 °C corresponds to a destabilization per porphyrin of only $\Delta T_{m,P} = -2.6$ °C. The interlocking stacking of the porphyrins thus has a substantial effect on the stability of the duplex. The melting profile showed a rather large hysteresis with $T_m = 64.3$ °C for the melting and $T_m = 57.7$ °C for the annealing (Figure 1a), which indicates quite different kinetics in the two processes.[17]

As we know from previously synthesized strands, the attachment of TPP in **2** onto one DNA strand leads to a decrease in the T_m of $\Delta T_m = -13.5$ °C for three porphyrins, or $\Delta T_m = -34.1$ °C for eleven porphyrins compared to the corresponding natural DNA strand in a 21 base-pair (bp)

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Table 1: Melting temperatures of the zipper porphyrin–DNA strands.[a]

DNA strands[b]	T_m [°C]	ΔT_m [°C]	$\Delta T_{m,P}$ [°C][c]
3a·3a	71.4		
3b·3b	61.0	−10.4	−2.6
4a·5a	64.5		
4b·5b	50.4	−4.1	−1.4
6a·7a	61.5		
6b·7b	67.2	+5.7	+0.5
7b·8b	60.6	−0.9	−0.1

[a] Thermal denaturation measurements were performed in 100 mM NaCl, 50 mM KH_2PO_4, pH 7.0, $c_{DNA} = 10^{-6}$ M. [b] For sequences see Scheme 1. [c] Change in thermal stability per porphyrin.

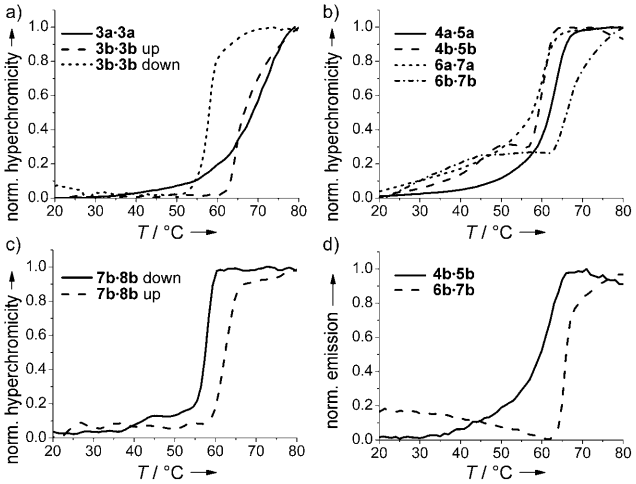

Figure 1. Melting profiles of the zipper porphyrin–DNA: a) melting and annealing of the DPP–DNA array **3b·3b** and of the unmodified DNA **3a·3a**; b) UV-melting of the TPP-DNA **4b·5b** and **6b·7b** and of the unmodified DNA; c) melting and annealing of TPP/DPP–DNA **7b·8b**; d) fluorescence melting curves (λ_{ex} 420 nm, λ_{em} 654 nm) of **4b·5b** and **6b·7b**. Conditions as in Table 1. "Up" denotes heating, "down" denotes cooling in the melting–annealing cycle.

sequence.[8] The zipper array **4b·5b**, incorporating three stacked TPP moieties after hybridization, displays $\Delta T_m = -4.1$ °C, that is, an effective ΔT_m of +9.4 °C for the zipper in comparison with the one-strand porphyrin–DNA. The effect is even more remarkable in the duplex **6b·7b**. In this case, we recorded an actual increase in melting temperature of $\Delta T_m = +5.7$ °C compared to the unmodified DNA, giving rise to an effective thermal stabilization of $\Delta T_m \approx +40$ °C in comparison to DNA with only one porphyrin-modified strand. The fluorescence melting of the TPP-zipper strands confirms the T_m values (see the Supporting Information). Upon excitation at 420 nm, the porphyrin emission at 654 nm increases with temperature, which is indicative of unstacking of the porphyrins during melting.

Arrays of the **2**-containing zipper show a rather sharp transition during melting compared to normal DNA. In addition, in the duplexes **4b·5b** and **6b·7b**, additional weak and broad transitions can be detected at lower temperatures. These low-temperature transitions at around 44 °C (**4b·5b**) and 35 °C (**6b·7b**) could be explained as fraying of the flanking sequences of the porphyrin-modified region, and are at higher temperatures than would be expected for the melting of the corresponding 12 bp (37 °C) and 8 bp (29 °C) sequences, based on calculations. These transitions are not detected by the fluorescence melting, thus are not associated with structural changes in the porphyrin modified region.

Results analogous to those described above could be obtained with the mixed porphyrin duplex **7b·8b**. The estimated T_m of this duplex is about 18 °C (based on an average drop in thermal stability of −7 °C (per molecule) for **1** and −3 °C for **2**). However, the actual melting temperature in the mixed porphyrin zipper array is $T_m = 60.6$ °C, showing a very large stabilization effect resulting from the stacking of the porphyrins, which corresponds to an increase of about 42 °C in thermal stability, as would be expected for DNA with only one porphyrin-modified strand. Again, the melting profile shows quite a large hysteresis, similar to the duplex **3b·3b**, with $T_m = 62.7$ °C for the melting and $T_m = 58.4$ °C for the annealing.

The UV region of the circular dichroism (CD) spectra shows that the zipper arrays adopt a B-type helical structure, as demonstrated by the characteristic bisignate signal having maxima at (−252)/(+277) nm (Figure 2a). The porphyrin B-band CD signals give rise to a negative peak, as previously recorded, and for the DPP-containing arrays this peak is broad and weak. The UV/Vis spectra confirm a stacked arrangement, as evidenced by broadening of the porphyrin B-band absorbance at ambient temperature (Figure 2b). When

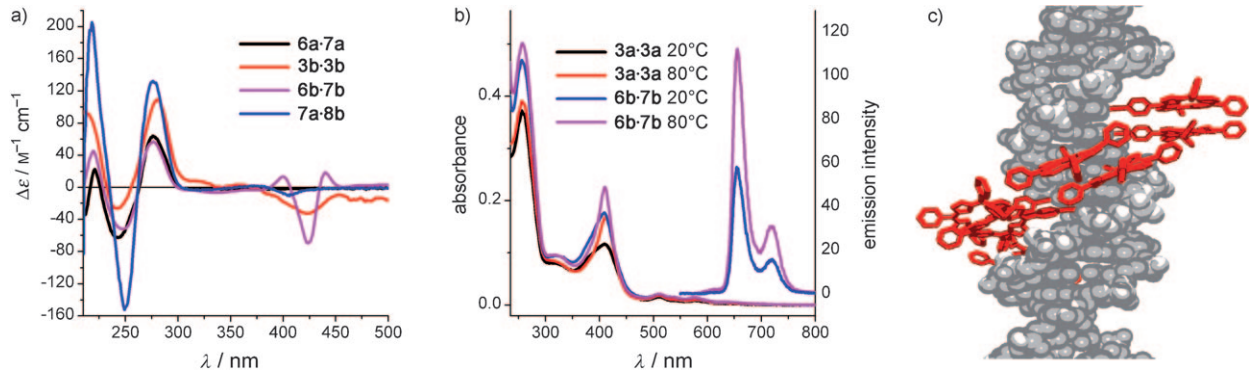

Figure 2. a) Selected CD spectra of DNA and zipper porphyrin arrays (20 °C); b) selected UV/Vis and emission spectra of porphyrin arrays at 20 °C and 80 °C; c) part of the calculated structure of **6b·7b** (MacroModel, Amber*). Conditions for spectroscopic measurements as in Table 1.

the double-stranded DNA is heated to 80 °C, the absorbances of the porphyrins sharpen significantly, indicating unstacking of the zipper arrays. Analogous spectral changes were reported by Endo, Fujitsuka, and Majima.[9] The same behavior is found with respect to the fluorescence of the porphyrins; unstacking at higher temperature leads to an increase of the fluorescence intensity, suggesting that the porphyrins do interact electronically when stacked in the zipper arrays within the major groove of the DNA.

The spectra are in accordance with the modeled structure of **6b·7b** (Figure 2c).[18] The porphyrins are located—as expected—in the major groove of the DNA. However, unlike arrays with all porphyrins attached to only one strand, leading to a contiguous stacking of the porphyrins, in this case, the porphyrins seem to be arranged in a zigzag fashion, and stack in pairs involving one group from each strand. This is a consequence of the different orientation of the substituents in the major groove on being placed on opposite strands.

A general problem in the automated synthesis of porphyrin–DNA, in which the porphyrins are located within the DNA sequence, is demetalation during the acidic removal of the dimethoxy trityl (DMT) protecting group. The porphyrin–DNA is obtained as the free base with complete loss of zinc from the porphyrin. In this manner, it is rather difficult to create mixed-metal porphyrin arrays. Recently, Berova and co-workers[19] reported remetalation of porphyrins on DNA with Cu and Zn. We also find it straightforward to reinsert metals into the porphyrin after DNA synthesis. The attachment of the porphyrins onto different strands now allows for the reinsertion of zinc into one of the porphyrin–DNA strands while the other strand is left metal-free. Metalation of **5b** and **7b** with $Zn(OAc)_2$ in buffered solution was readily achieved to give **Zn-5b** and **Zn-7b**. The metalation was monitored by absorbance and fluorescence spectroscopy. The fluorescence spectra of the mixed porphyrin arrays **4b·Zn-5b** and **6b·Zn-7b** revealed partial quenching of the fluorescence of the zinc porphyrin in the annealed DNA and an enhanced fluorescence of the free-base porphyrin (Figure 3). Upon denaturing of the zipper array, the relative fluorescence of the zinc-metalated porphyrin–DNA strand increased, and the emission spectrum of the final completely dissociated system could be reproduced by a superposition of the spectra of the individual strands. In the array **4b·Zn-5b** the relative emission of the zinc porphyrin increased substantially more than that of the free-base porphyrin upon unstacking. In the array **6b·Zn-7b**, however, the relative emission of the free-base porphyrin actually decreased upon melting, indicating a much larger energy-transfer efficiency in the longer porphyrin array, which overcompensates the stacking-induced quenching of the free-base porphyrin. Similar resonance energy transfer between zinc and free-base porphyrins based on a non-covalent DNA–porphyrin assembly have been reported recently by D'Souza et al.[20] The zinc metalation does not alter the thermal stability, and the DNA duplexes **4b·Zn-5b** and **6b·Zn-7b** show the same T_m values as the free-base analogues, as determined by fluorescence melting of the arrays.

In summary, the attachment of large hydrophobic substituents onto complementary strands of DNA led to a zipper array, where—in contrast to previously reported small-molecule systems—the hydrophobic interactions could lead to a substantial stabilization of the DNA duplex, compared to single-strand modification. More than four porphyrin moieties were required to obtain stabilization. The porphyrins tended to stack very efficiently in the major groove of the DNA, leading to stable helical multiporphyrin arrays. The overall effect was influenced by the structure of the porphyrin but not by its metalation state. The complementary strand could be remetalated separately to give mixed zinc-metalated and free-base porphyrin arrays, which showed energy transfer between the porphyrins when annealed but not when denatured. To our knowledge, this result represents the first case of up to eleven porphyrins arranged in a stacked supramolecular structure, selectively and reversibly, through DNA annealing or melting. Our system gives relatively easy access to mixed porphyrin systems, for example, in terms of substitution pattern or metalation state, both of which are important in the design of electronic or photonic wires. We are now exploring the electron- and energy-transfer efficiencies of our systems.

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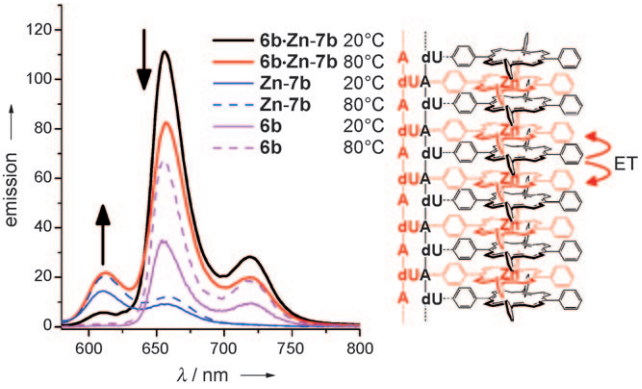

Figure 3. Emission spectra of the free-base and zinc-metalated strands **6b** and **Zn-7b** (20 °C), and of the mixed metal–porphyrin array **6b·Zn-7b** at 20 °C (annealed) and 80 °C (denatured). ET = energy transfer.

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