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PAPER

The DNA three-way junction as a mould for tripartite chromophore assembly†

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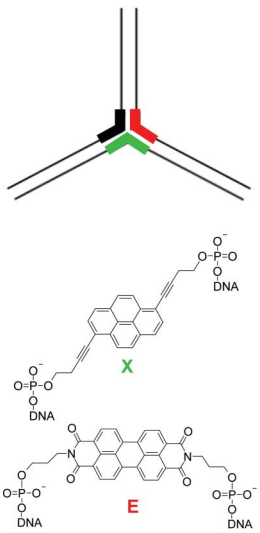
The DNA three-way junction serves as a scaffold for the molecular organization of non-nucleosidic alkynylpyrene and perylenediimide chromophores located at the branch point of the structure. Depending on the composition of the tripartite assembly, the constructs possess distinct spectroscopic properties, ranging from monomer or excimer fluorescence to completely quenched tripartite aggregates.

Introduction

The use of nucleic acids for the controlled arrangement of functional molecules has emerged as an important research area.[1–7] The hybridization of modified oligonucleotides allows the precise placement of functionalities along the double helix. Single, double and multistranded hybrids have been used for the generation of a considerable diversity of constructs.[8–13] The molecular interaction of individual chromophores can be directed by the formation of different types of hybrid structures, which opens up possibilities for diagnostic, electronic, optical and mechanical applications.[14–25] We recently reported on the DNA guided generation of alkynylpyrene–perylenediimide assemblies in single and double stranded hybrids.[26–28] The spectroscopic properties of these aggregates are largely controlled by the nature of the interstrand stacking interactions of the chromophores.[28–30] The diversity of interactions of individual chromophores may be increased by using various types of nucleic acids structures, such as the three-way junction (3WJ). In this common, naturally occurring junction, a branch point is formed by three DNA or RNA stems.[31–36] The application of a DNA 3WJ as a yoctoliter-scale reactor[37] and as a structure-switching module in DNA nanodevices[38] have been reported recently. Here, we report the use of this higher order DNA structure as a scaffold for assembling individual chromophoric units in the branch point area.

Results and discussion

The design of the study is illustrated in Scheme 1. The 3WJ is formed by three oligodeoxynucleotides that are partially complementary in a pairwise fashion. Alkynylpyrene (**X**) and perylenediimide (**E**) building blocks are positioned in the middle of the oligomers so that they are located at the branch point of the formed 3WJ. The modified oligomers were synthesized following the procedures described previously.[28,39] In combination with the unmodified control oligodeoxynucleotides these oligomers were used to form a total of 14 different 3WJs, **3WJ-1** to **3WJ-8** are shown in Scheme 1, whereas **3WJ-9** to **3WJ-14** are listed in the ESI.† The length and nucleotide sequence of the oligomers were chosen with help of the *UNAFold* software[40,41] to ensure sufficient stability of the tripartite hybrids at room temperature avoiding the formation of other, unwanted secondary structures. Additionally, direct neighboring guanines next to the modification were avoided in order to minimize fluorescence quenching by this base. **3WJ-1** and **3WJ-2**, which serve as controls, are 'perfectly paired'[33] or contain an extra thymine base in each strand at the branch point.



		Sequence (5'-3')
3WJ-1	(a)	GAA GGA ACG T-A CAC TCG CAG
	(b)	GTT CCA CGC T-A CGT TCC TTC
	(c)	CTG CGA GTG T-A GCG TGG AAC
3WJ-2	(d)	GAA GGA ACG TTA CAC TCG CAG
	(e)	GTT CCA CGC TTA CGT TCC TTC
	(f)	CTG CGA GTG TTA GCG TGG AAC
3WJ-3	(g)	GAA GGA ACG TXA CAC TCG CAG
	(b)	GTT CCA CGC T-A CGT TCC TTC
	(h)	CTG CGA GTG TXA GCG TGG AAC
3WJ-4	(g)	GAA GGA ACG TXA CAC TCG CAG
	(e)	GTT CCA CGC TTA CGT TCC TTC
	(h)	CTG CGA GTG TXA GCG TGG AAC
3WJ-5	(g)	GAA GGA ACG TXA CAC TCG CAG
	(i)	GTT CCA CGC TXA CGT TCC TTC
	(h)	CTG CGA GTG TXA GCG TGG AAC
3WJ-6	(g)	GAA GGA ACG TXA CAC TCG CAG
	(j)	GTT CCA CGC TEA CGT TCC TTC
	(h)	CTG CGA GTG TXA GCG TGG AAC
3WJ-7	(k)	GAA GGA ACG TEA CAC TCG CAG
	(i)	GTT CCA CGC TXA CGT TCC TTC
	(l)	CTG CGA GTG TEA GCG TGG AAC
3WJ-8	(k)	GAA GGA ACG TEA CAC TCG CAG
	(j)	GTT CCA CGC TEA CGT TCC TTC
	(l)	CTG CGA GTG TEA GCG TGG AAC

Scheme 1 Schematic illustration of the 3WJs investigated (**3WJ-1** to **3WJ-8**) and structures of the non-nucleosidic building blocks 1,6-di-alkynylpyrene (**X**) and perylenediimide (**E**).

Formation of 3WJs by the oligomers was initially tested with polyacrylamide gel electrophoresis (PAGE). Fig. 1 shows a selected set of single strands and hybrids. According to their migration

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† Electronic supplementary information (ESI) available: Synthetic and analytical details; additional UV/Vis, fluorescence and CD spectra; polyacrylamide gels, molecular models. See DOI: 10.1039/c1ob06400b

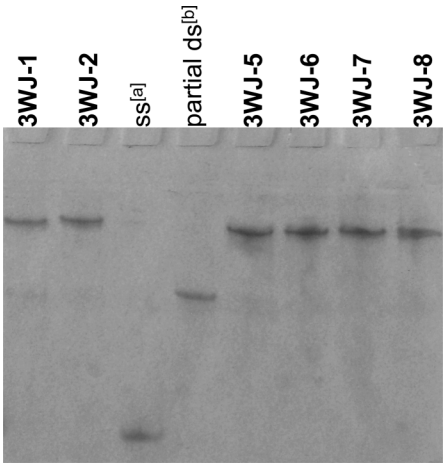

Fig. 1 Non-denaturing polyacrylamide gel of unmodified and chromophore-modified DNA 3WJs; [a] single strand **h**; [b] mixture of two single strands (**h** and **i**). Conditions: 4 μM single strand concentration, 90 mM Tris-borate buffer, pH 8.0, 4 °C.

behaviour, all different sets form 3WJs under the conditions applied (see also ESI†). No substantial differences in migration of dye-modified and unmodified hybrids could be observed, ruling out the potential formation of larger aggregates, *e.g.* due to interactions of individual 3WJs through dye–dye interactions. Thermal denaturation experiments (see ESI†) revealed for all hybrids a single, sharp transition, indicating a two-step process. In case of the reference systems (**3WJ-1** and **3WJ-2**), the melting temperature (T_m) values were in the range of 43–44 °C, whereas all 3WJs containing modifications showed considerably higher thermal stabilities (*ca.* +15 °C, see ESI†).

Information on the interactions between the individual chromophores was obtained from UV/Vis spectroscopy. Aggregation behaviour is well illustrated by **3WJ-5** (Fig. 2) showing the changes in the vibronic band intensity ratios that are characteristic for alkynylpyrene aggregation (330–410 nm region).[39] At 20 °C the $A^{0\rightarrow1}$ transition (368 nm) is of higher intensity than the $A^{0\rightarrow0}$ transition (389 nm) indicating aggregation. At 90 °C the intensities are reversed, as expected for monomer-like alkynylpyrenes.

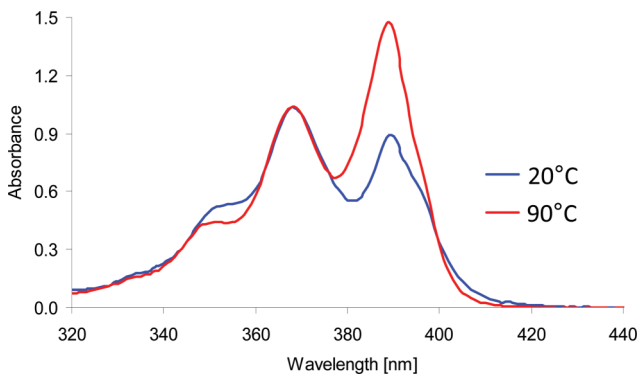

Fig. 2 Temperature-dependent UV/Vis spectra of **3WJ-5** with three alkynylpyrenes; normalized at the $A^{0\rightarrow1}$ transition band. Conditions: 1 μM single strand concentration, 100 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0.

The thermally induced aggregation–deaggregation process observed in the absorption spectrum goes in parallel with the fluorescence properties shown for the same hybrid in Fig. 3. At 20 °C **3WJ-5** exhibits nearly exclusively excimer behaviour (emission maximum around 526 nm), whereas at 90 °C only monomer emission is detected.[42–52] The formation of alkynylpyrene excimers is, thus, enabled by bringing together the individual alkynylpyrene residues at the branch point of the 3WJ. In the dissociated state the alkynylpyrenes do not interact and monomer fluorescence is observed exclusively.

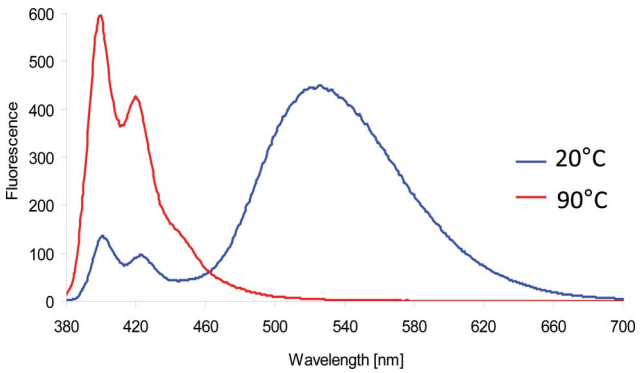

Fig. 3 Temperature-dependent fluorescence spectra of **3WJ-5** containing three alkynylpyrenes. Conditions: see Fig. 2. Instrumental set-up: λ_{ex}: 370 nm, excitation slit width: 5 nm; emission slit width: 5 nm; PMT voltage: 600 V.

In addition to thermal denaturation, the alkynylpyrene monomer–excimer ratio is also influenced by removing one alkynylpyrene unit (**3WJ-3**), or replacing it with one thymine base (**3WJ-4**) or, most effectively, with one perylenediimide unit (**3WJ-6**). This is illustrated in Fig. 4, displaying the normalized spectra of these three combinations. In **3WJ-3** the branch point contains two alkynylpyrenes that can aggregate in a dimer-like structure, resulting in a reduced excimer fluorescence compared to the above described **3WJ-5**. In junction **3WJ-4** the extra thymine base may be additionally involved in a separation of the alkynylpyrenes leading to a further reduction of excimer formation. Introduction of one perylenediimide unit (**3WJ-6**) was previously shown to severely prevent alkynylpyrene excimer formation ***via*** formation of a donor–acceptor–donor (D–A–D) complex.[28,53–56] The deaggregation of the two alkynylpyrenes and the resulting loss of excimer emission, due to insertion of a perylenediimide unit, is

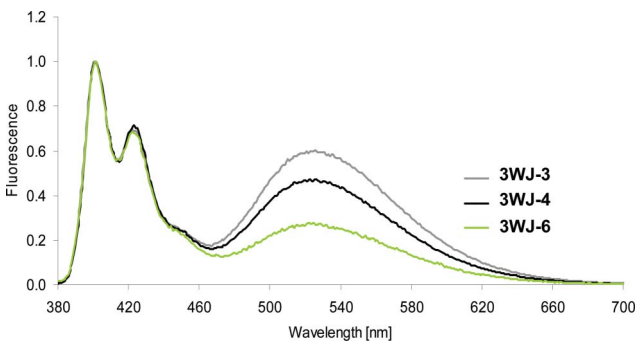

Fig. 4 Fluorescence spectra of alkynylpyrene containing junctions (**3WJ-3**, **3WJ-4** and **3WJ-6**) normalized at 400 nm (20 °C). Conditions and instrumental set-up: see Fig. 3.

also supported by changes of the $A^{0\rightarrow0}/A^{0\rightarrow1}$ ratios in the UV/Vis spectra (see ESI†).

An assembly containing two perylenediimide units and one alkynylpyrene at the branch point (**3WJ-7**) shows associative behaviour of both building blocks. The UV/Vis spectra taken at 20 and 90 °C (Fig. 5) show the well known change in the ratio of the vibronic bands of the perylenediimide (440–600 nm) associated with the aggregation–deaggregation process. In addition, both chromophores exhibit a significant bathochromic shift (**X**: 7 nm, **E**: 8 nm) in their respective absorption bands upon aggregation which serves as evidence for the formation of a stable ground-state complex of the three chromophores.

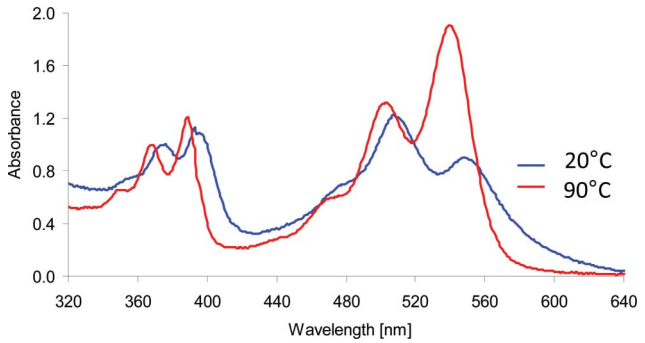

Fig. 5 Temperature-dependent UV/Vis spectra of the **3WJ-7**, normalized on the $A^{0\rightarrow1}$ band of the alkynylpyrene building block; **X**: 330–410 nm, **E**: 440–600 nm. Conditions: see Fig. 2.

Experiments performed with the junction assembling three perylenediimide building blocks at the branch point (**3WJ-8**) also showed a significant hypsochromic shift upon thermal denaturation (Fig. 6) along with the familiar change in the vibronic band ratio associated with deaggregation.[57–63]

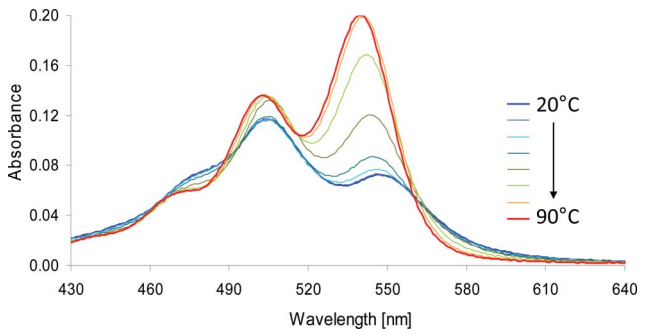

Fig. 6 Temperature-dependent UV/Vis spectra of **3WJ-8** with three perylenediimide units at the branch point. Conditions: see Fig. 2.

The effect of perylenediimide on the fluorescence properties of alkynylpyrene is clearly documented by the series **3WJ-5**, **3WJ-6** and **3WJ-7** (Fig. 7). This series illustrates the effects resulting from stepwise replacement of an alkynylpyrene by a perylenediimide. Introduction of a single perylenediimide in the assembly leads to a dramatic reduction of alkynylpyrene excimer fluorescence with little monomer fluorescence remaining. This observation is closely related to the one made in a duplex system, for which a sandwich type arrangement (**X–E–X**), *i.e.* the perylenediimide stacked between two alkynylpyrenes, was derived.[28] Based on the close similarity of those and the present data, this type

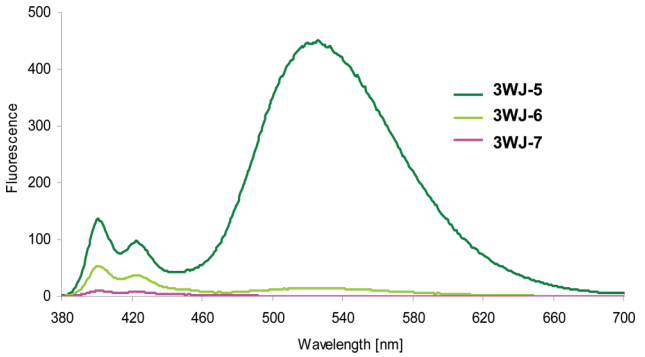

Fig. 7 Fluorescence emission spectra of **3WJ-5**, **3WJ -6** and **3WJ-7**. Conditions and instrumental set-up: see Fig. 3.

of chromophore arrangement is most likely also present in **3WJ-6**. Additional alkynylpyrene *vs.* perylenediimide substitution (**3WJ-7**) also results in a near complete quenching of the monomer fluorescence.

Circular dichroism (CD) spectroscopy indicates, for both types of chromophores, exciton coupling. This is best observed in the 3WJs containing only one kind of modification, **3WJ-5** and **3WJ-8** (Fig. 8). The amplitude is much more pronounced for the perylenediimide than for the alkynylpyrene, suggesting that the perylenediimide is involved in tighter intermolecular interactions than the alkynylpyrene. Nevertheless, both chromophores are arranged in twisted stacking interactions.

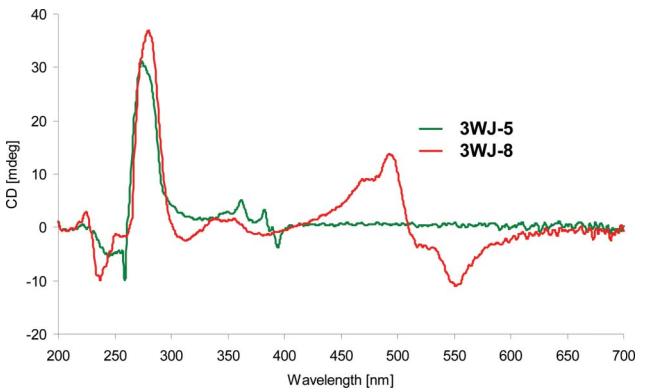

Fig. 8 CD spectra of the two homo-chromophoric 3WJs. **3WJ-5** (three alkynylpyrenes) and **3WJ-8** (three perylenediimides). Conditions: 5 μM single strand concentration, 100 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0.

Taking the evidence provided by the experiments described above, the following conclusions can be drawn: i) the 3WJ provides an excellent framework for assembling non-nucleosidic chromophoric building blocks; ii) perylenediimide and/or alkynylpyrene chromophore arrays formed at the branch point of the tripartite complex fit excellently with the general rules observed in duplex based systems described for these chromophores; iii) perylenediimide and alkynylpyrene building blocks are involved in stacking interactions, as suggested by UV/Vis and CD spectroscopy, and iv) based on fluorescence data it can be concluded that, in mixed aggregates, a donor–acceptor–donor (*e.g.* **X–E–X**) stacking interaction is predominant.[64] Molecular modelling[65] of **3WJ-6**, which contains two alkynylpyrenes and one

perylenediimide, was performed in an attempt to visualize these conclusions. Depending on the constraints made for optimization of the structure, three different types of branch structures (see also ESI†) were obtained. One of these, shown in Fig. 9, is compatible with the essential elements described, *i.e.* the chromophores are arranged in an alternating sequence (**X–E–X**) in a twisted π-stack at the branch point of the three-way junction. Two of the DNA stems are arranged in a linear fashion, while the third stem is oriented in a 90 ° angle, forming a T-shaped structure. The inner (hydrophobic) cavity provides the environment for the chromophore array.

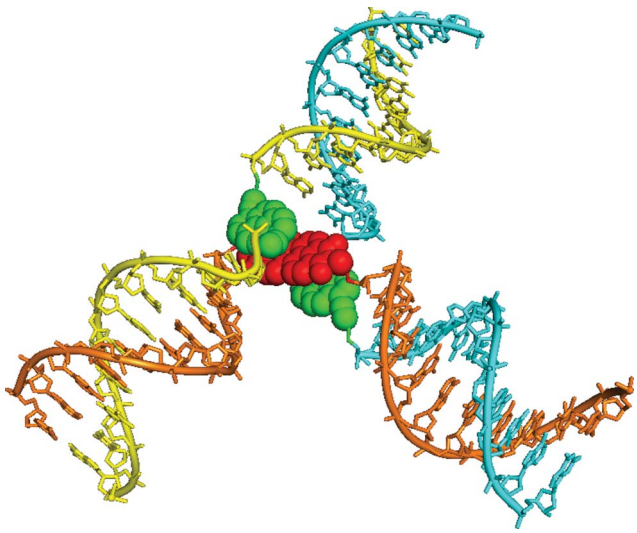

Fig. 9 Energy-minimized model of the three-way junction **3WJ-6** containing two alkynylpyrenes (green) and a perylenediimide (red) forming a π-stacked aggregate at the branch point.

Conclusions

The present study shows that the DNA 3WJ serves as a suitable scaffold for the assembly of non-nucleosidic chromophores. Alkynylpyrene and perylenediimide building blocks form stable aggregates at the branch point of the 3WJ. Tight interactions between the individual chromophores lead to stabilization of the 3WJ. Furthermore, the hybrids exhibit spectroscopic properties that are characteristic for molecular aggregates. Absorption and fluorescence behaviour can be controlled by the choice of the chromophore combination, ranging from monomer and excimer fluorescence to completely quenched tripartite aggregates. The data demonstrate the value of higher order DNA structures for the molecular assembly of functional molecules.

Experimental section

The two phosphoramidite building blocks, 1,6-dialkynylpyrene (**X**)[39] and perylenediimide (**E**)[66] were synthesized according to previously described protocols. Modified oligomers were prepared by automated oligonucleotide synthesis using an adapted synthetic procedure on a 394-DNA/RNA synthesizer (Applied Biosystems).[28] Cleavage from the CPG solid support (GlenResearch, Sterling, USA) and the final deprotection were done by treatment with a 30% solution of NH_4OH in a thermomixer (Eppendorf) at 55 °C overnight. Purification was performed by RP-HPLC (LC-10AT, Shimadzu; LiChrospher 100 RP-18 5 µm, Merck, Darmstadt, Germany). Unmodified oligodeoxynucleotides were obtained from Microsynth (Balgach, Switzerland). Oligonucleotide masses were determined by LC-MS (negative ion mode, acetonitrile/H_2O/triethylamine) on a Sciex QTrap (Applied Biosystems).

UV/Vis spectra were taken on a Varian Cary-100 Bio-UV/Vis spectrophotometer equipped with a Varian Cary-block temperature controller and processed with Varian WinUV software. CD spectroscopy was performed on a JASCO J-715 spectrophotometer using 1 cm quartz cuvettes. Fluorescence spectroscopy involved the use of a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Varian Cary-block temperature controller with 1 cm × 1 cm quartz cuvettes and Varian Eclipse software. Instrumental setups for fluorescence emission spectra were: λ_{ex}: 370 nm; excitation slit width: 5 nm; emission slit width: 5 nm; PMT voltage: 600 V. The concentration of the single strands was adjusted to 1 µM (except for CD measurements, in which a 5 µM oligomer solution was used). Additionally, each solution contained 10 mM sodium phosphate buffer, pH 7.0 and 100 mM NaCl.

Polyacrylamide gel electrophoresis (PAGE) was performed using a 10% stacking gel on top of a 20% resolving gel (approx. 10 cm length). 2 µL of loading buffer (33% glycerol in Tris-borate buffer) were added to 8 µL of sample (final oligomer concentration: 4 µM each strand; 90 mM Tris-borate buffer, pH 8.0) and the mixture was loaded onto the gel. After running the gel (170V/6mA/2W) for 2 h in a closed chamber at 4 °C, gels were stained with Stains-all reagent dissolved in a buffered formamide solution.

Acknowledgements

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64 It is noteworthy that many additional combinations with the strands listed in Scheme 1 exist, which are included in the ESI.† Although slight quantitative differences may be found, all fit very well qualitatively into the picture given by the sets described in the main text.
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