A modular LHC built on the DNA three-way junction†

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A light-harvesting complex composed of a π-stacked multichromophoric array in a DNA three-way junction is described. The modular design allows for a ready exchange of non-covalently attached energy acceptors.

In attempts to mimic the naturally occurring process of light harvesting and energy transfer in green plants,1–5 scientists are developing artificial systems6–17 that allow an efficient transfer of absorbed photons to a collection centre. A key aspect in all efforts is the proper arrangement of suitable chromophores. The field of DNA nano-architectures has received wide attention over the past years. The DNA double helix serves as a scaffold for the controlled arrangement of functional molecules,18–23 including a wide range of different chromophores.24–32 Also the DNA three-way junction33 has been exploited for this purpose.34–42 Recently, several DNA-based light-harvesting antenna systems have been reported.43–51 Here, we describe the use of the DNA three-way junction (3WJ) as a versatile scaffold for the modular construction of an artificial light harvesting complex (LHC).

The architecture of the 3WJ, its sequences and the corresponding building blocks are shown in Scheme 1. The phenanthrene (P),52 pyrene (S and X)53,54 perylenediimide (E),55 and the commercially available cyanine (Cy) phosphoramidite building blocks were incorporated into the oligomers by automated oligonucleotide synthesis. All oligomers were purified by reverse phase HPLC and analysed by LC-MS (see ESI†). In 3WJ-2, -3, -4 and -5, the LHC is composed of a phenanthrene (P) stack, the light absorbing antenna (strands a and b), and an exchangeable acceptor chromophore (strands d-g). 3WJ-1 serves as a control bearing no acceptor dye in the third strand (c). Formation of the 3WJs was demonstrated by polyacrylamide gel electrophoresis (ESI†). Thermal denaturation experiments showed a single transition for all 3WJs. Melting temperature (T\text{\textsubscript{m}}) values ranging from 57–63 °C are listed in Table 1. UV/Vis spectra of the different 3WJs (Fig. 1) reveal the following long-wavelength absorptions of the various chromophores: P (\(\lambda\text{\textsubscript{max}} = 316\) nm); S (\(\lambda\text{\textsubscript{max}} = 353\) nm); X (\(\lambda\text{\textsubscript{max}} = 391\) nm); E (\(\lambda\text{\textsubscript{max}} = 561\) nm) and Cy (\(\lambda\text{\textsubscript{max}} = 652\) nm).

The effectiveness of the 3WJs as LHCs was investigated by fluorescence spectroscopy. Fig. 2 shows the emission spectra of 3WJ-1 to 3WJ-4 after excitation of phenanthrene at 320 nm. 3WJ-2 and 3WJ-3, both of which contain a pyrene derivative as acceptor, exhibit strong
exciplex ($\lambda_{\text{max}} = 435$ and 455 nm, respectively$^{56}$). This is in agreement with the observation made previously with a duplex containing a phenanthrene antenna with a covalently attached pyrene (S).$^{46}$ In the present case, however, the acceptor dye is brought into proximity of the phenanthrene antenna in a non-covalent fashion.

A quantum yield of 0.21 (Table 1) is obtained for 3WJ-2. Similar results are obtained for 3WJ-3. However, some pyrene monomer fluorescence (peak at 400 nm and shoulder at around 425 nm) is present in addition to the exciplex emission. The total quantum yield of 3WJ-3 with 1,6-dialkylpyrene as the acceptor dye is 0.32.

Irradiation of the control 3WJ-1, which contains the phenanthrene stack but no acceptor chromophore, at 320 nm results in a broad, unstructured emission band centred around 415 nm, which is assigned to phenanthrene excimer fluorescence.$^{57-60}$ $\Phi_F$ for 3WJ-1 was 0.10 (Table 1). This is considerably less than the values found for 3WJ-2 and -3 (0.21 and 0.32) that contain a terminal pyrene chromophore.

In three-way junction 3WJ-4, a perylenediimide unit is used in strand c. This chromophore quenches the phenanthrene excimer fluorescence to a considerable extent ($\Phi_F$ 0.02). The quenching effect$^{61,62}$ is strong, but not as pronounced as in the case of pyrene excimer quenching by the same perylenediimide derivative.$^{37,63}$ The reduced effect can be rationalized by a smaller spectral overlap in the present case (phenanthrene–perylene-
diimide) than between pyrene and perylenediimide.$^{63}$

The emission spectra obtained by direct excitation of the two
pyrene acceptor dyes (S or X) are shown in Fig. 3. Excitation was carried out at 370 nm, at which wavelength both pyrene derivatives absorb and phenanthrene excitation is negligible. Two aspects are worth noting: (i) the fluorescence intensities are significantly lower than that obtained after excitation of phenanthrene, which shows that the phenanthrene–pyrene 3WJ functions as a LHC; and (ii) the fraction of monomer emission in 3WJ-3 is somewhat larger than after phenanthrene excitation (cf. Fig. 2). Furthermore, direct excitation of control 3WJ-1 at 370 nm (Fig. 3) results in no significant fluorescence.

Energy transfer is also observed from phenanthrenes to Cy. Fig. 4 shows the effect of a stepwise build-up of 3WJ-5. In this experiment, the phenanthrene antenna (i.e. a 1:1-mixture of strands a and b) is added stepwise to the acceptor dye (strand g). A gradual increase in Cy fluorescence is observed until antenna and acceptor are present in equimolar concentrations. Phenanthrene excimer emission is also present and grows parallel to Cy fluorescence. Further addition of the antenna beyond the 1:1-ratio does not result in additional growth of Cy fluorescence, while phenanthrene fluorescence grows proportionally. The quantum yields for P and Cy emission amount to 0.015 and 0.049, respectively (Table 1). Cy has no significant absorbance (see ESI† Fig. S28) at the irradiation wavelength (320 nm) or in the region of phenanthrene emission (360–530 nm). As shown by the corresponding excitation spectra (Fig. 5), phenanthrene excitation (320 nm) leads to Cy emission only in 3WJ-5 but not in single strand g. These experiments demonstrate that Cy fluorescence in 3WJ-5 is due to energy transfer from the phenanthrenes.$^{64}$ A Förster radius of $\sim 26 \text{ Å}$ is calculated (ESI†). Considering the dimensions of the cavity in a typical 3WJ ($\sim 12 \text{ Å}$)$^{65}$ as well as its inherent flexibility,$^{3,65}$ energy transfer via FRET is, thus, possible. On the other hand, molecular contact between the donor and the acceptor is also

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**Fig. 1** UV/Vis spectra of the investigated three-way junctions. Conditions: 1 µM single strand, 100 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0; acceptors are given in brackets.

**Fig. 2** Fluorescence emission spectra of 3WJ-1, 3WJ-2, 3WJ-3 and 3WJ-4. Conditions: see Fig. 1; $\lambda_{\text{exc}}$: 320 nm; ex/em. slit: 5 nm.

**Fig. 3** Fluorescence spectra of 3WJ-1, 3WJ-2 and 3WJ-3. Conditions: see Fig. 1. Instrumental set-up as in Fig. 2 (except for $\lambda_{\text{exc}}$: 370 nm).

**Fig. 4** Build-up of 3WJ-5: fluorescence spectra after stepwise addition of strands a and b (1:1-mixture) to single strand g. The two strands were pre-annealed and added in 0.2 molar equivalents: an equilibration time of 5 minutes was given before recording the spectrum; other conditions were as shown in Fig. 2. Dashed marker indicates artefacts caused by the second order transmission of the monochromator.
Fig. 5 Fluorescence excitation spectra of 3WJ-5 and single strand g.
Conditions: Fig. 1; instrumental set-up: Fig. 2, except for λem: 670 nm; dashed marker: see Fig. 4.

feasible (as e.g. shown by exciplex formation in 3WJ-2 and -3) rendering energy transfer via Dexter electron exchange a plausible mechanism.

In conclusion, we have described the construction of LHCs on the basis of the DNA 3WJ. The LHC is based on a modular construction in which a phenanthrene antenna is located in one of the three stems and the acceptor is brought into proximity of the antenna through the annealing of the third strand. Phenanthrene excitation (320 nm) is followed by energy transfer to pyrene, perylenediimide or a cyanine dye. In the case of pyrene, exciplex fluorescence is observed, whereas perylenediimide acts as a quencher. In the case of cyanine, energy transfer leads to cyanine fluorescence. The study demonstrates the use of the DNA 3WJ for the modular assembly of artificial LHCs.

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Notes and references

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