Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

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

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Electronic Supplementary information

(ESI)

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Synthetic and analytical procedure

The building blocks alkynylpyrene (X)[1] and perylenediimide (E)[2] were synthesized as previously described. The unmodified strands were obtained commercially from Microsynth, Balgach, Switzerland. The modified strands were prepared via automated oligonucleotide synthesis by an adapted synthetic procedure on a 394-DNA/RNA synthesizer (Applied Biosystems)[3]. Cleavage from the solid support and final deprotection was done by treatment with 30% NH_4OH solution at 55°C overnight.

Purification was performed by reverse phase HPLC (LiChrospher 100 RP-18, 5 um, Merck; Shimadzu LC-20AT and Kontron Instruments). Mass spectrometry was done with LC-MS (negative ion mode, acetonitrile/H_2O/triethylamine) on a Sciex QTrap (Applied Biosystems).

Temperature-dependent UV/Vis spectra were carried out on a Varian Cary-100 Bio-UV/Vis spectrophotometer equipped with a Varian Cary-block temperature controller and data were collected with Varian WinUV software, over the range of 200-700 nm at 10-90°C in 10°C increments. CD spectra were recorded on a JASCO J-715 spectrophotometer using quartz cuvettes with an optic path of 1 cm. Fluorescence spectra were performed on a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Varian Cary-block temperature controller using 1 cm x 1 cm quartz cuvettes.

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) was 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 2h in a closed chamber at 4°C, gels were stained with Stains-all reagent dissolved in a buffered formamide solution.

[1] H. Bittermann, D. Siegemund, V. L. Malinovskii, R. Häner, *J.Am.Chem.Soc.*, 2008, **130**, 15285-15287.
[2] N. Rahe, C. Rinn, T. Carell, *Chem.Commun.*, 2003, 2119-2121.
[3] S. M. Biner, D. Kummer, V. L. Malinovskii, R. Häner, *Org.Biomol.Chem.*, 2011, **9**, 2628-2633.

Investigated oligonucleotides and masses

The sequences were purified with RP-HPLC chromatography, using a gradient of 5 – 55 % MeCN over 20 min, starting from 100 % Et_3NH / Acetic acid (0,1 M, pH 7,4). In case of the three strands containing a PDI unit, a gradient of 5 – 40 % was applied and the elution was performed at 60°C.

Table S1: Molecular formula, masses, retention times and epsilons.

Sequence (5´-3´)	*molecular formula*	*calcd. avg. mass*	*found avg. mass*	t_R *[min]*	*calcd.* ε $[dm^3\ mol^{-1}\ cm^{-1}]$
GAA GGA ACG T**X**A CAC TCG CAG	C219H260N84O117P20	6560,4	6560,0	12,2	252300
GTT CCA CGC T**X**A CGT TCC TTC	C216H265N63O127P20	6395,3	6395,4	11,9	207900
CTG CGA GTG T**X**A GCG TGG AAC	C220H262N80O122P20	6598,4	6598,0	12,4	240400
GAA GGA ACG T**E**A CAC TCG CAG	C225H264N86O121P20	6728,5	6727,1	18,5	258000
GTT CCA CGC T**E**A CGT TCC TTC	C222H269N65O131P20	6563,4	6562,0	17,0	213600
CTG CGA GTG T**E**A GCG TGG AAC	C226H266N82O126P20	6766,5	6765,5	17,4	246100

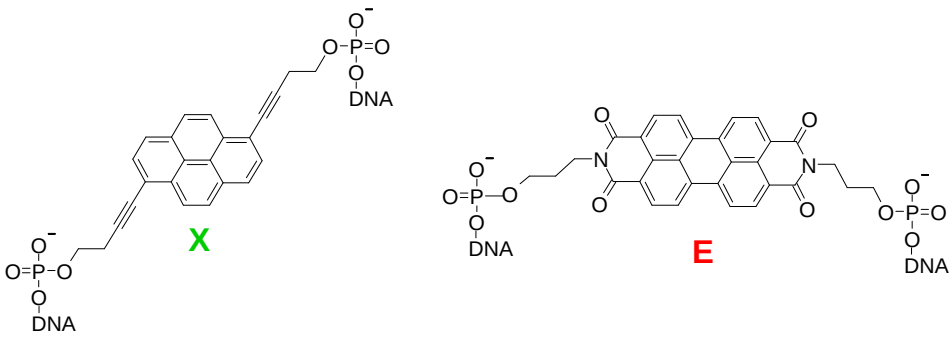

Fig. S1: Structures of the non-nucleosidic building blocks 1,6-dialkynylpyrene (**X**) and PDI (**E**).

Overview three-way junctions

Table S2: Summary and abbreviations of all the samples (5´-3´).

Name	Sequence (5´-3´)	Name	Sequence (5´-3´)
3WJ-1	GAA GGA ACG T-A CAC TCG CAG GTT CCA CGC T-A CGT TCC TTC CTG CGA GTG T-A GCG TGG AAC	**3WJ-8**	GAA GGA ACG TEA CAC TCG CAG GTT CCA CGC TEA CGT TCC TTC CTG CGA GTG TEA GCG TGG AAC
3WJ-2	GAA GGA ACG TTA CAC TCG CAG GTT CCA CGC TTA CGT TCC TTC CTG CGA GTG TTA GCG TGG AAC	**3WJ-9**	GAA GGA ACG TXA CAC TCG CAG GTT CCA CGC TXA CGT TCC TTC CTG CGA GTG TEA GCG TGG AAC
3WJ-3	GAA GGA ACG TXA CAC TCG CAG GTT CCA CGC T-A CGT TCC TTC CTG CGA GTG TXA GCG TGG AAC	**3WJ-10**	GAA GGA ACG TEA CAC TCG CAG GTT CCA CGC TXA CGT TCC TTC CTG CGA GTG TXA GCG TGG AAC
3WJ-4	GAA GGA ACG TXA CAC TCG CAG GTT CCA CGC TTA CGT TCC TTC CTG CGA GTG TXA GCG TGG AAC	**3WJ-11**	GAA GGA ACG TEA CAC TCG CAG GTT CCA CGC TEA CGT TCC TTC CTG CGA GTG TXA GCG TGG AAC
3WJ-5	GAA GGA ACG TXA CAC TCG CAG GTT CCA CGC TXA CGT TCC TTC CTG CGA GTG TXA GCG TGG AAC	**3WJ-12**	GAA GGA ACG TXA CAC TCG CAG GTT CCA CGC TEA CGT TCC TTC CTG CGA GTG TEA GCG TGG AAC
3WJ-6	GAA GGA ACG TXA CAC TCG CAG GTT CCA CGC TEA CGT TCC TTC CTG CGA GTG TXA GCG TGG AAC	**3WJ-13**	GAA GGA ACG TEA CAC TCG CAG GTT CCA CGC T-A CGT TCC TTC CTG CGA GTG TEA GCG TGG AAC
3WJ-7	GAA GGA ACG TEA CAC TCG CAG GTT CCA CGC TXA CGT TCC TTC CTG CGA GTG TEA GCG TGG AAC	**3WJ-14**	GAA GGA ACG TEA CAC TCG CAG GTT CCA CGC TTA CGT TCC TTC CTG CGA GTG TEA GCG TGG AAC

T_M values

Table S3: T_M of the individual three-way junctions. T_M of **3WJ-1 – 3WJ-8** were determined via temperature dependent UV-Vis spectra at 260 nm.

Name	T_m	**Name**	T_m
3WJ-1	44°C	**3WJ-5**	52°C
3WJ-2	43°C	**3WJ-6**	59°C
3WJ-3	47°C	**3WJ-7**	61°C
3WJ-4	45°C	**3WJ-8**	63°C

Thermal denaturation profiles

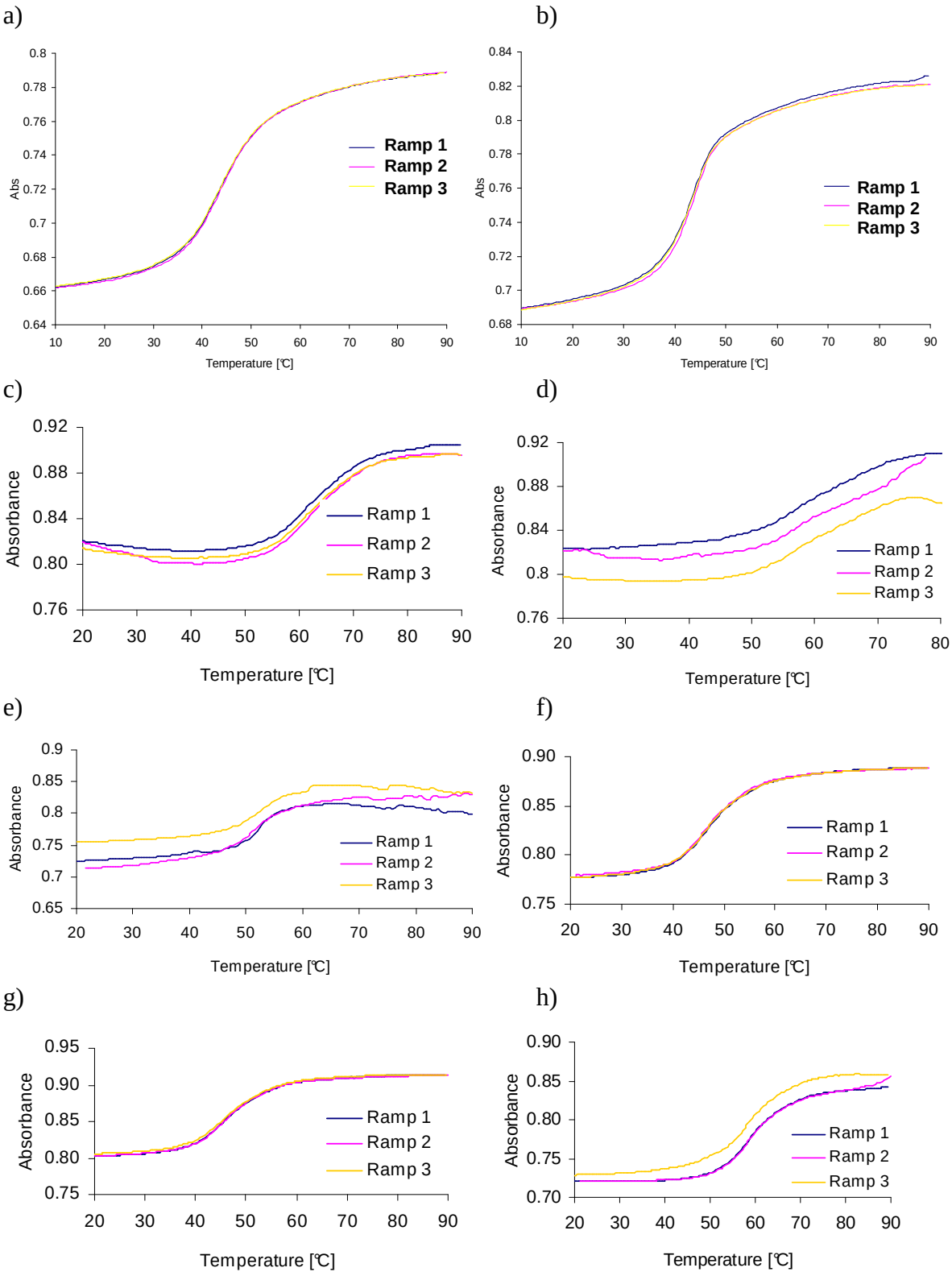

Fig. S2: Melting profile of **3WJ-1** (a), **3WJ-2** (b), **3WJ-7** (c), **3WJ-8** (d), **3WJ-5** (e), **3WJ-3** (f), **3WJ-4** (g) and **3WJ-6** (h). Conditions: 1.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. The absorption was measured at 260 nm. Ramp 1: 90°C → 10°C, Ramp 2: 10°C → 90°C and Ramp 3: 90°C → 10°C.

Temperature dependent UV/Vis measurements

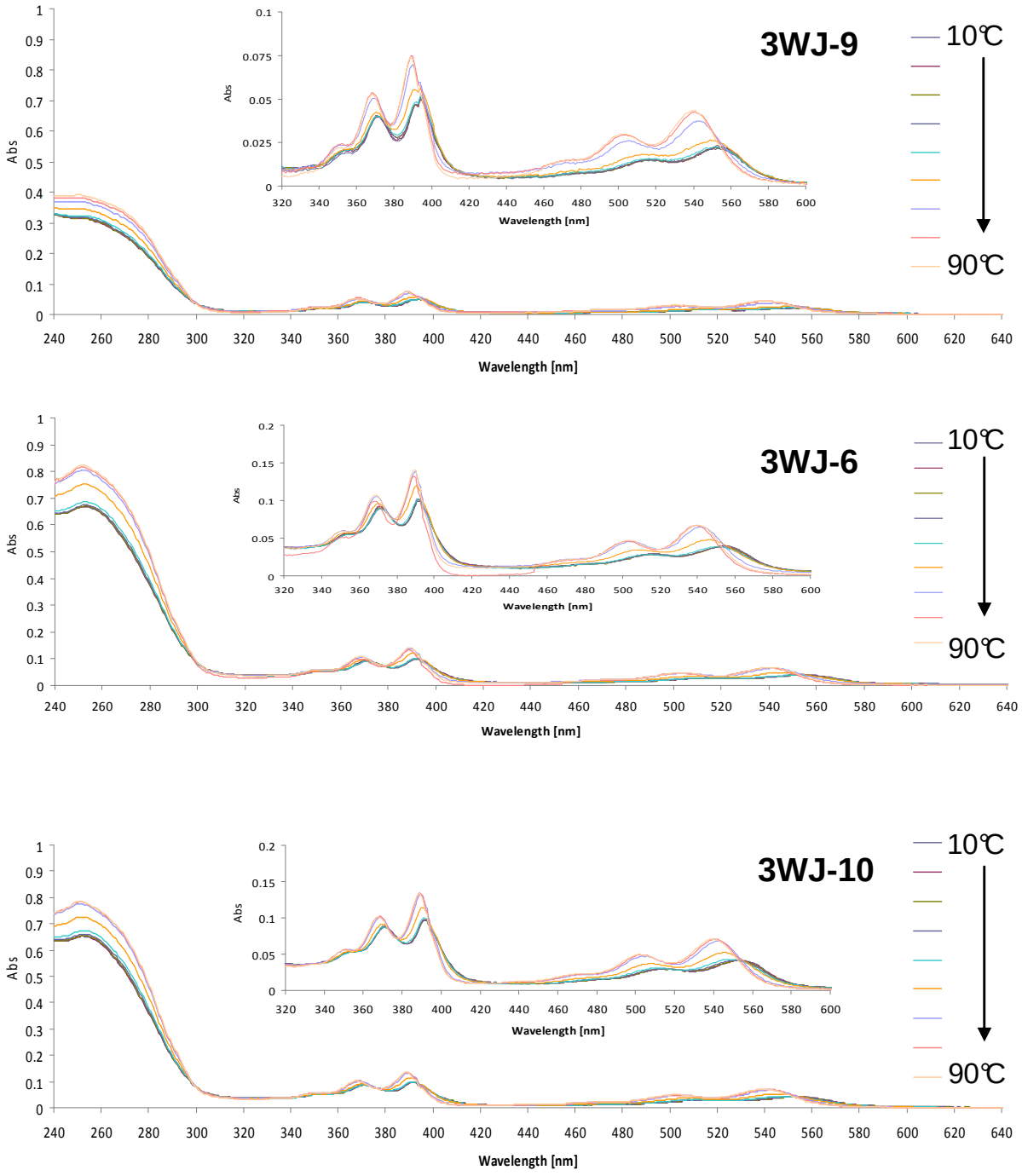

Fig. S3: Temperature dependent absorbance spectra. Conditions: Concentration of the samples: 1.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Samples were cooled down to 10°C and then heated up to 90°C in steps of 10°C (each time an equilibration time of 5 min was chosen).

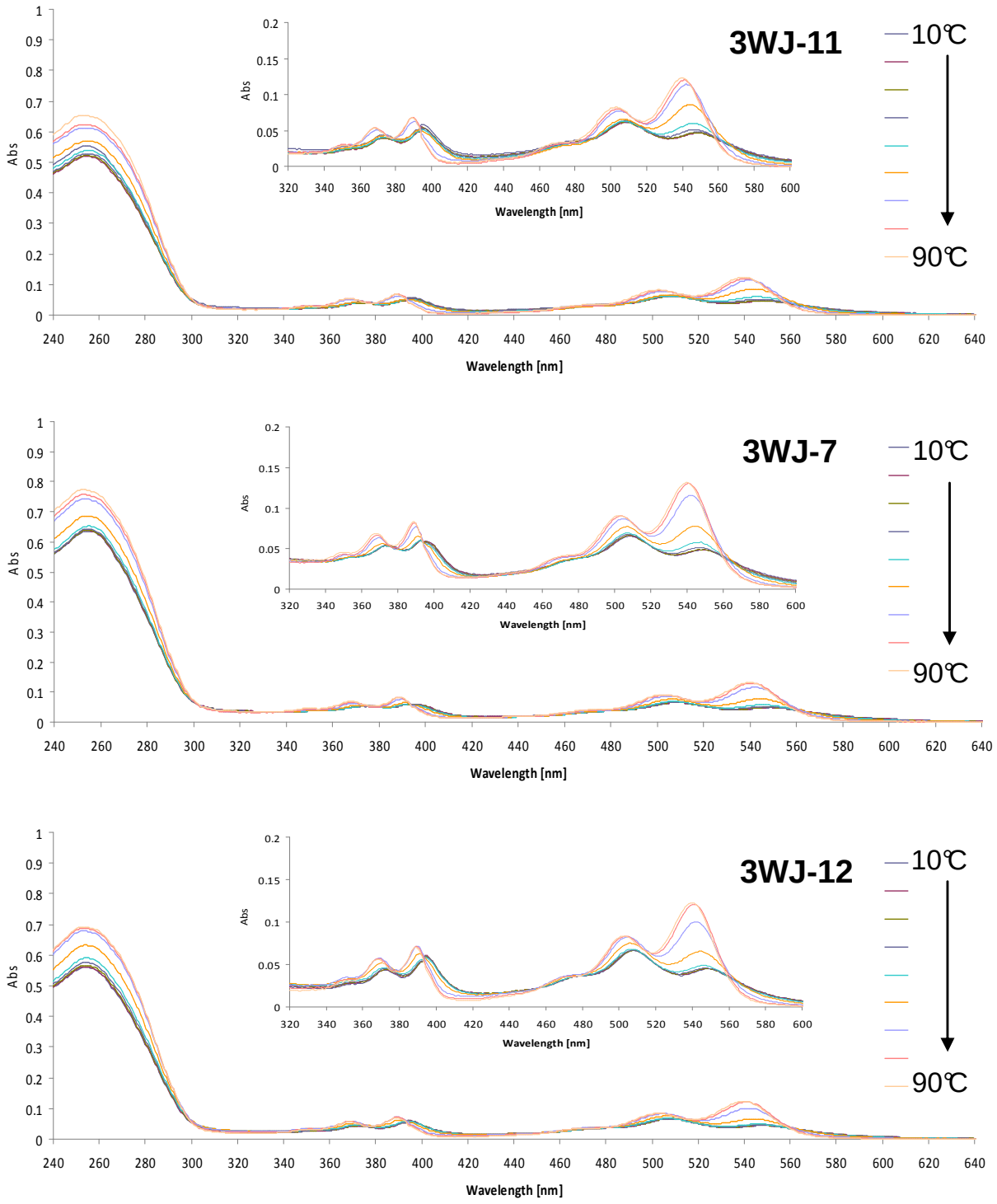

Fig. S4: Temperature dependent absorbance spectra. Conditions: Concentration of the samples: 1.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Samples were cooled down to 10°C and then heated up to 90°C in steps of 10°C (each time an equilibration time of 5 min was chosen).

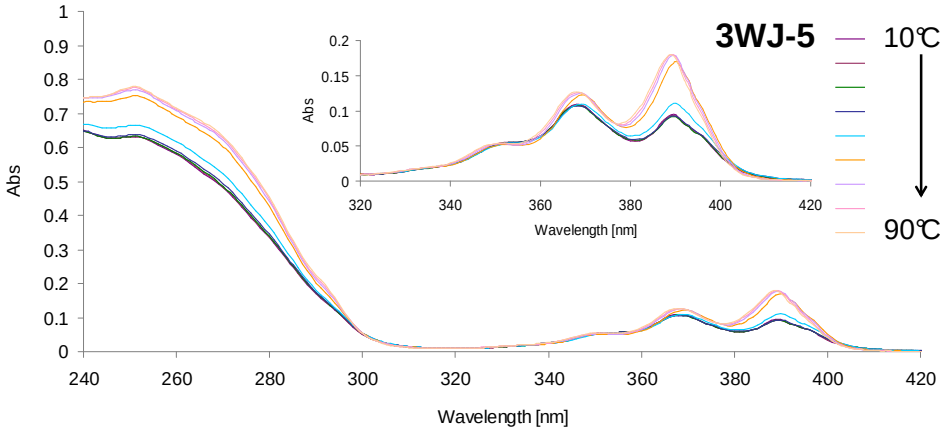

Fig. S5: Temperature dependent absorbance spectra. Conditions: Concentration of the samples: 1.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Samples were cooled down to 10°C and then heated up to 90°C in steps of 10°C (each time an equilibration time of 5 min was chosen).

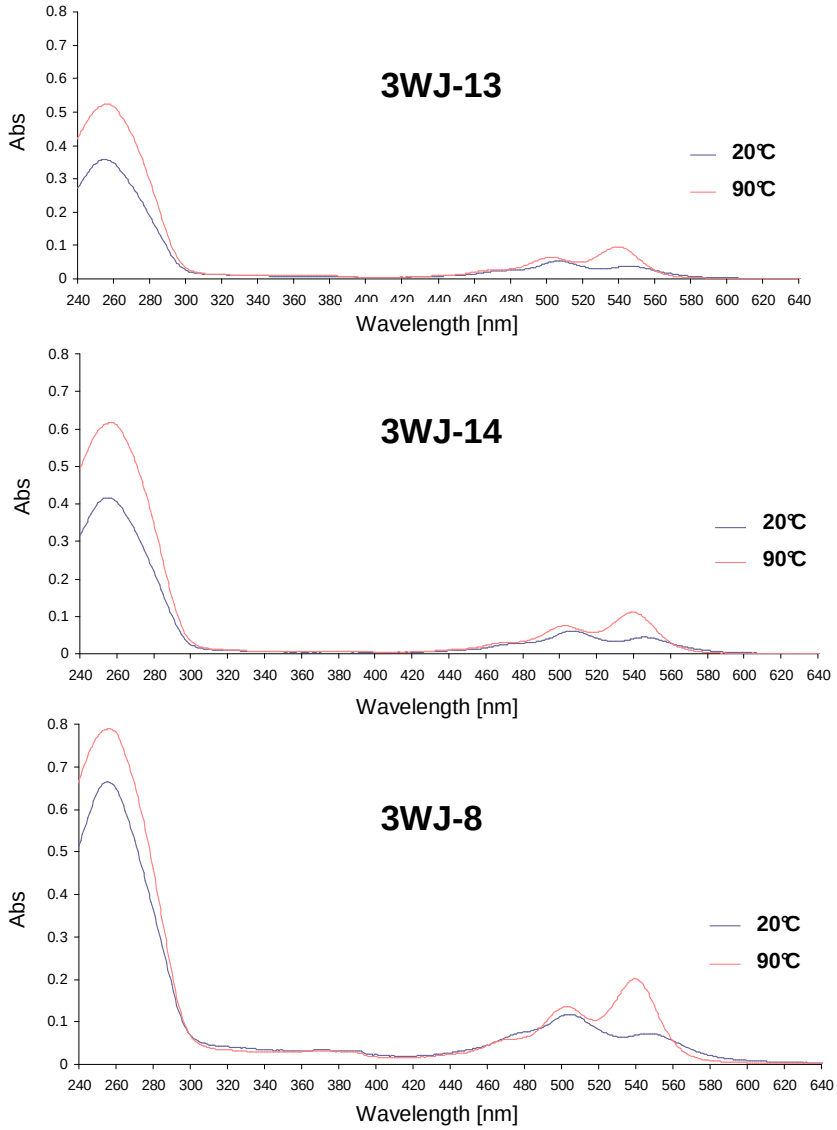

Fig S6: Temperature dependent absorbance spectra. Conditions: Concentration of the samples: 1.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl..

Fluorescence measurements: emission spectra

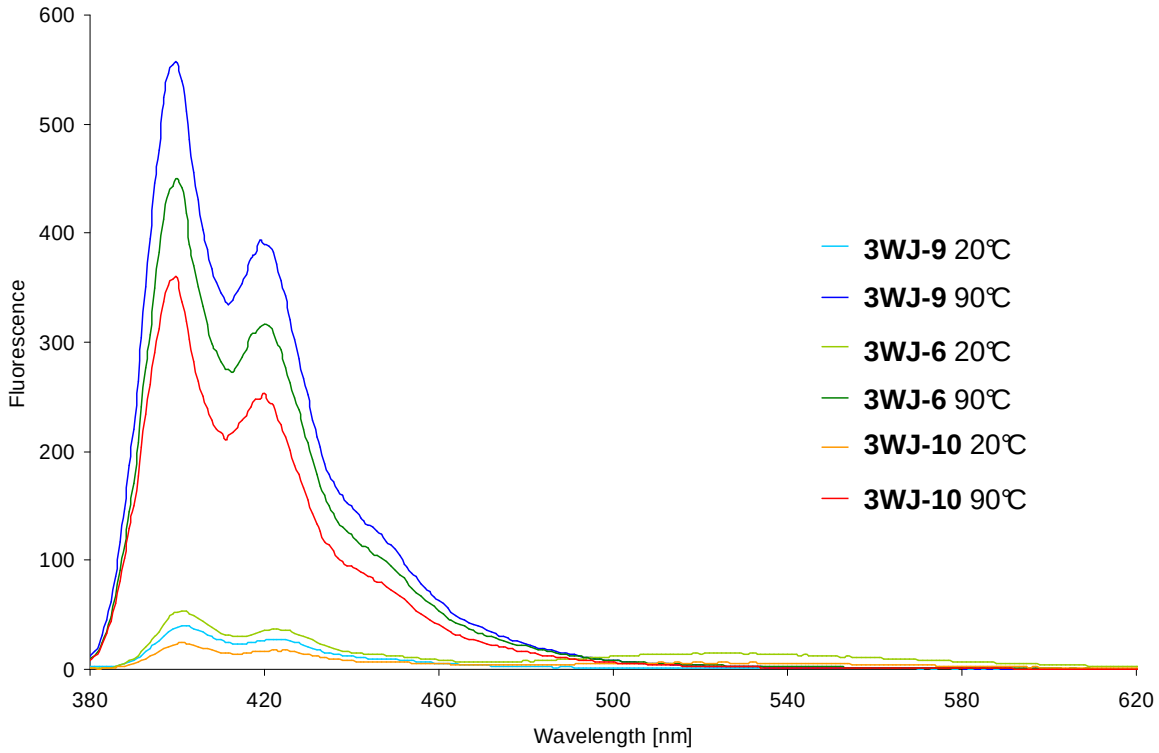

Fig. S7: Fluorescence spectra of the three samples that contain two alkynylpyrene building blocks and one PDI building block. The concentration of each three-way junction was adjusted to 1.0 µM. Conditions: 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Excitation at 370 nm, excitation slit width: 5 nm, emission slit width: 5 nm and PMT voltage: 600 V.

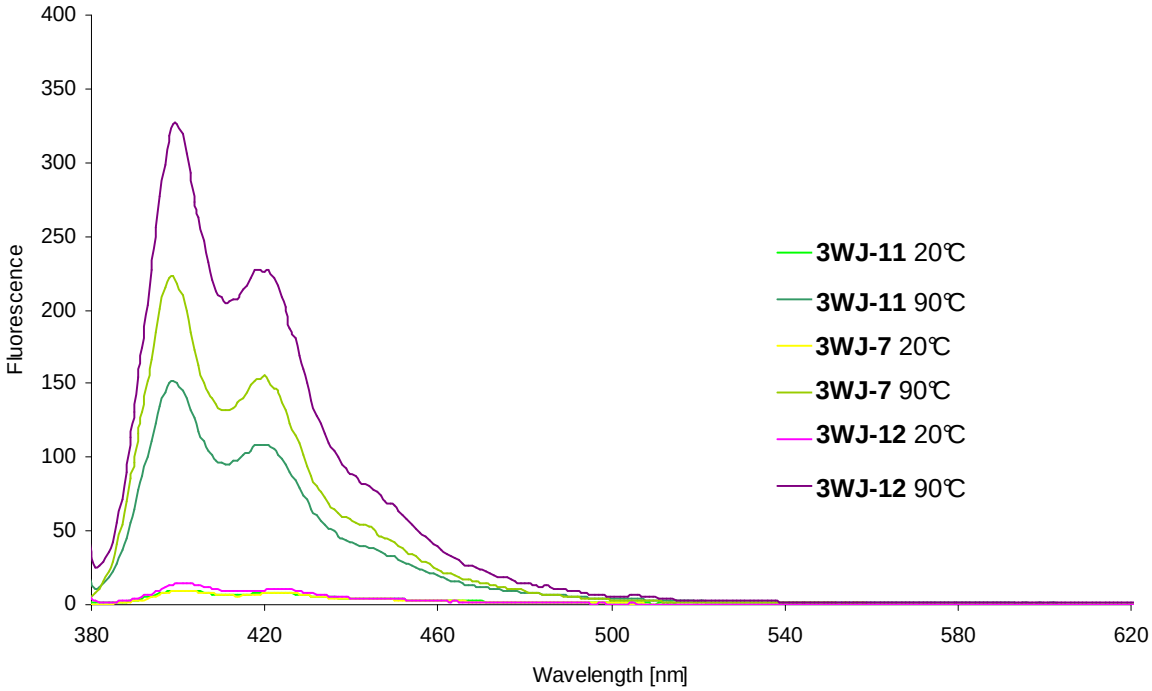

Fig. S8: Fluorescence spectra of the three samples that contain one alkynylpyrene building block and two PDI building blocks. The concentration of each three-way junction was adjusted to 1.0 µM. Conditions: 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Excitation at 370 nm, excitation slit width: 5 nm, emission slit width: 5 nm and PMT voltage: 600 V.

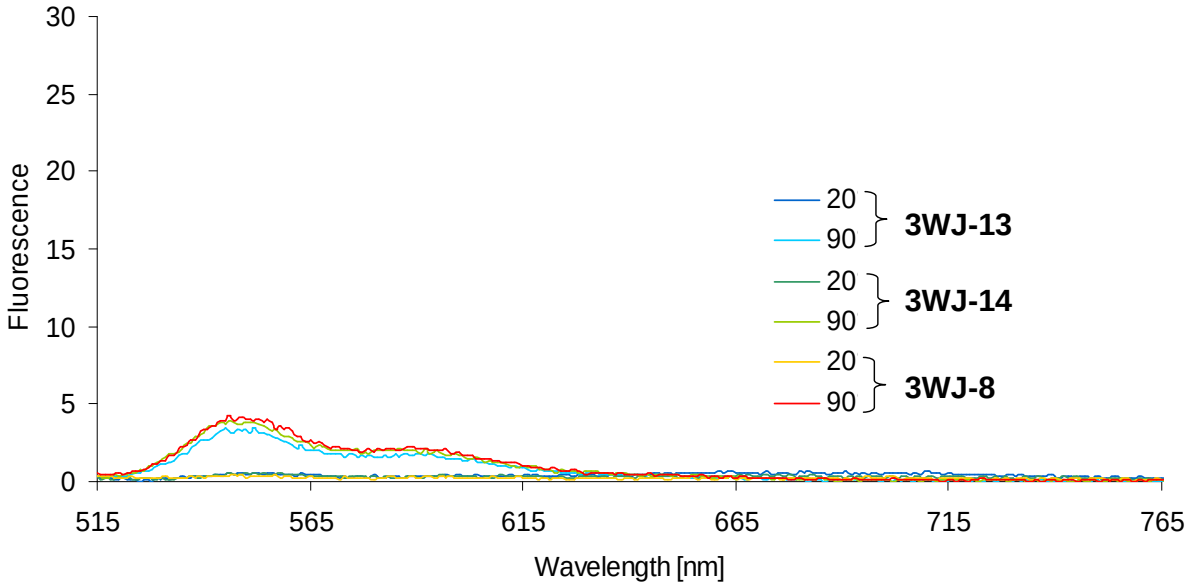

Fig. S9: The concentration of each three-way junction was adjusted to 1.0 µM. Conditions: 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Excitation at 505 nm, excitation slit width: 5 nm, emission slit width: 5 nm and PMT voltage: 600 V.

CD spectra

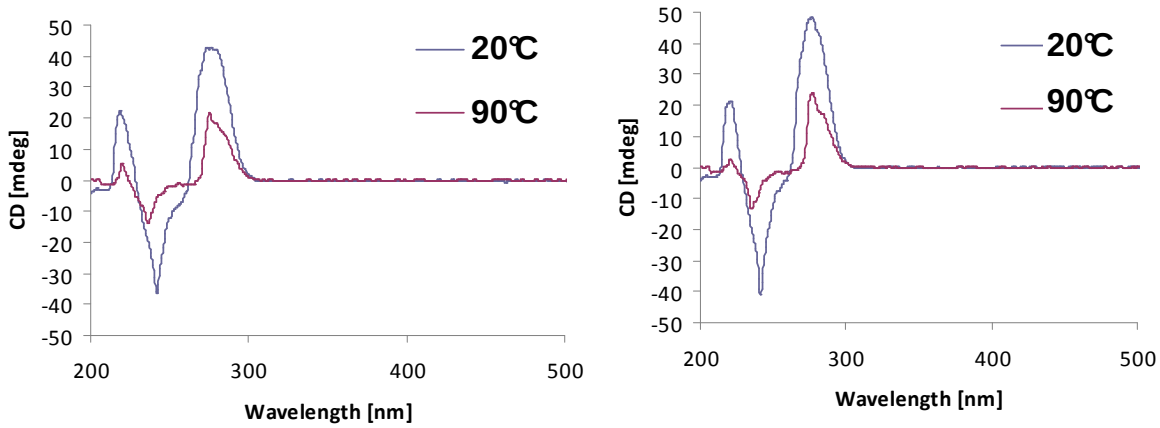

Fig. S10: CD spectra of **3WJ-1** (left) and **3WJ-2** (right). Conditions: Concentration of the samples: 5.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Samples were heated up to 90°C and cooled down to 20°C prior to the measurement. Starting temperature: 20°C, then increasing to 90°C (equilibration time: 10 min).

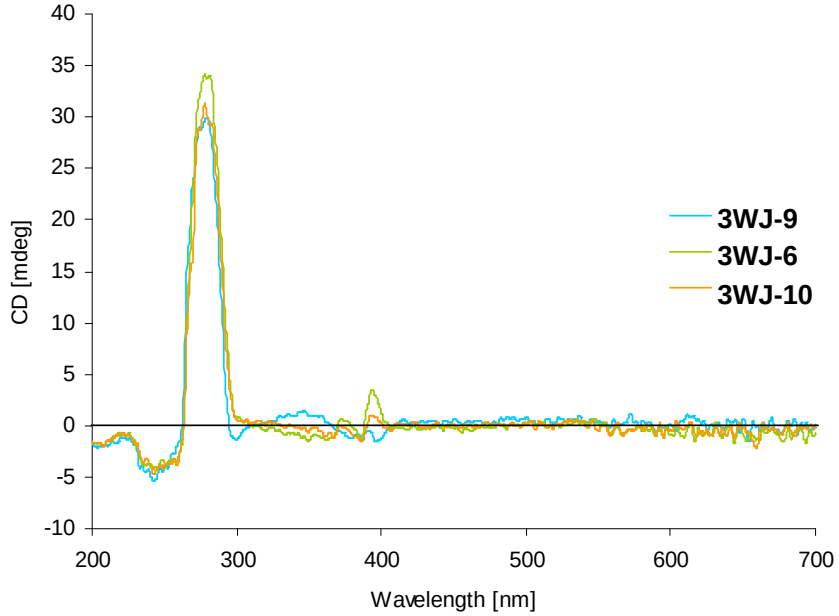

Fig. S11: CD spectra of three-way junctions containing two pyrenes and one PDI at the branch point. Conditions: Concentration of the samples: 5.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Samples were heated up to 90°C and slowly cooled down to 20°C prior to the measurement.

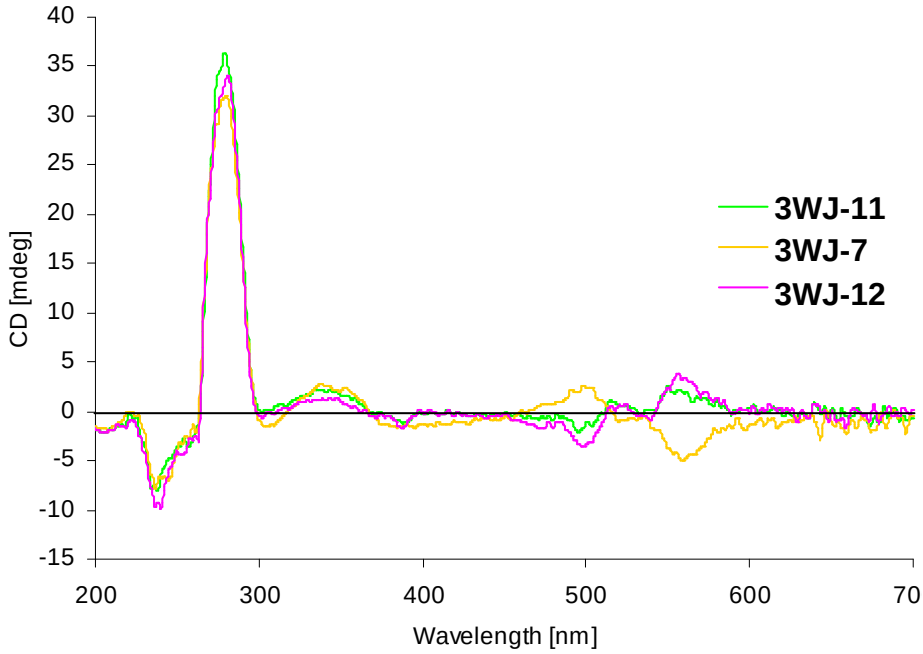

Fig. S12: CD spectra of three-way junctions containing two PDIs and one pyrene at the branch point. Conditions: Concentration of the samples: 5.0 µM, 10 mM phosphate buffer pH 7.0 and 100 mM NaCl. Samples were heated up to 90°C and slowly cooled down to 20°C prior to the measurement.

Additional polyacrylamide gels

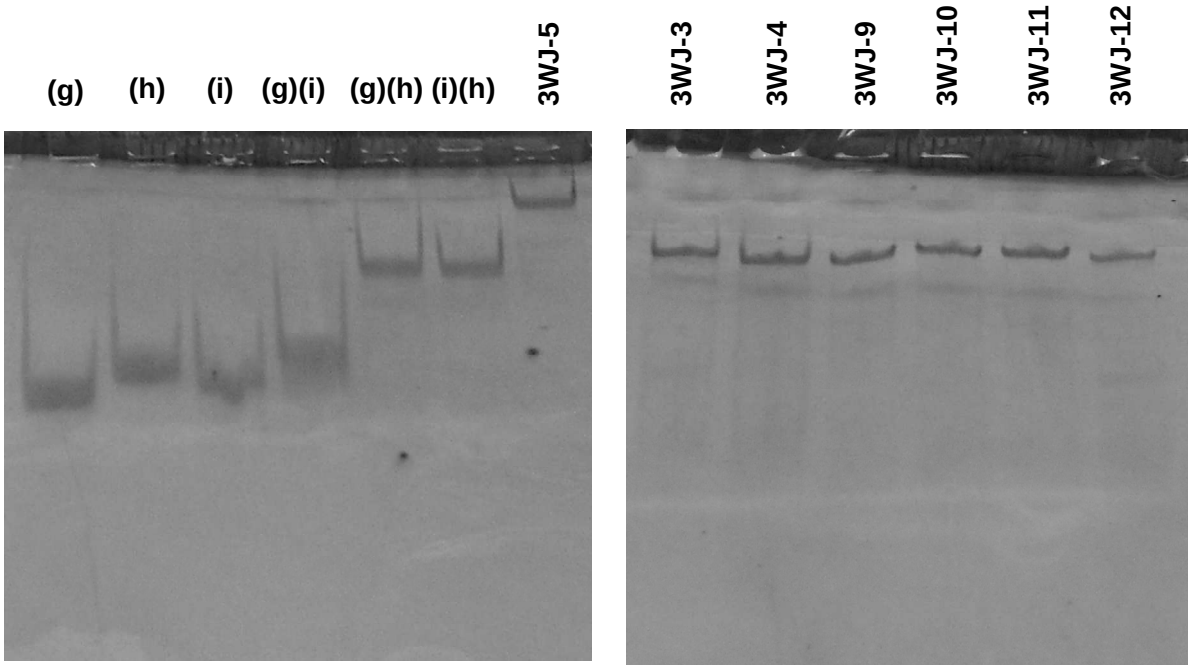

Fig. S13: 20% Non-denaturing polyacrylamide gels of three-way junctions. 2 µL of loading buffer (33% glycerol in Tris-borate buffer) was added to 8 µL of sample (final oligomer concentration: 4 µM each strand; 90 mM Tris-borate buffer, pH 8.0) and the mixture was loaded onto the gel.

Molecula Modeling of **3WJ-6**

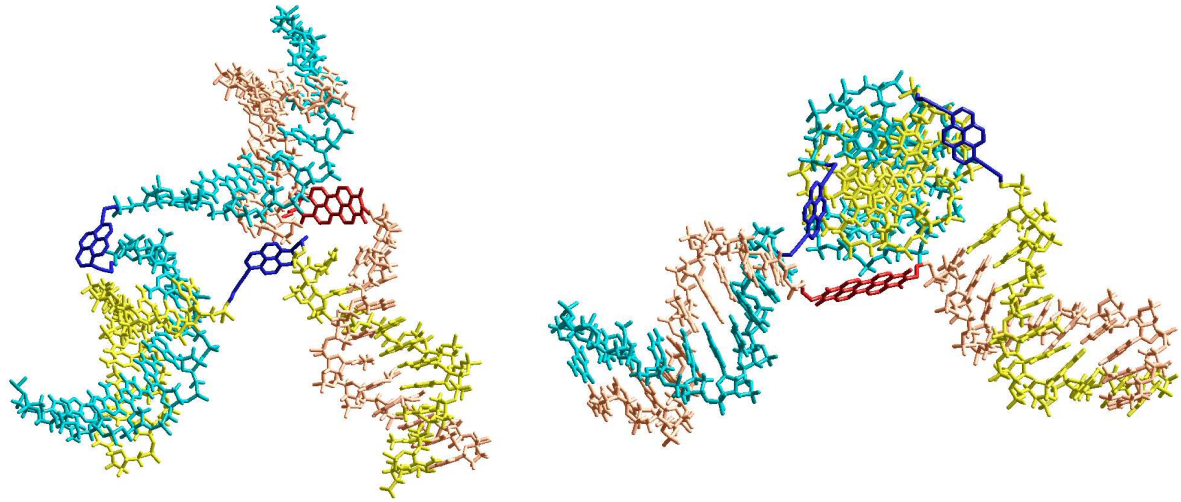

Fig. S14: Molecular models[4] of **3WJ-6**, which contains two pyrenes and one PDI. Depending on the constraints applied for optimization of the structure, two types of structures were obtained in addition to the one described in the main text. These are shown here. In the structure shown on top, chromophores are arranged on the 'outside' of the branch point area. In the minimum energy structure shown at the bottom, the three stems form a tripod shape; chromophores are located in the branch point area. Also in this structure, no direct interaction is present between the chromophores.

[4] HyperChem(TM), Hypercube, Inc., 1115 NW 4th Street, Gainesville, Florida 32601, USA; Release 8.0.8. 2010.