Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences

Ratiometric molecular beacons with absorption readout based on the perylene bisimide chromophore as an internal DNA base substitution

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Supporting Information

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Materials and Methods

Chemicals were purchased from Aldrich, Alfa Aesar and Merck. Unmodified oligonucleotides were purchased from Metabion. T.l.c. was performed on Fluka silica gel 60 F254 coated aluminum foil. Flash chromatography was carried out with silica gel 60 from Aldrich (60–43 μm). Spectroscopic measurements were recorded in Na-P_i buffer solution (10 mM) using quartzglass cuvettes (10 mm). Absorption spectra and the melting temperatures (2.5 μM DNA, 250 mM NaCl, 10–90 °C, 0.7 °C/min, step width 0.5 °C) were recorded with a Varian Cary 100 spectrometer equipped with a 6 x 6 cell changer unit. Fluorescence was measured with a Jobin–Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 6 nm and are corrected for Raman emission from the buffer solution.

Preparation of oligonucleotides

Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. For the PBI phosphoramidite the coupling time was enhanced from 96 s to 1500 s. To avoid precipitation during DNA synthesis, the PBI phosphoramidite was dissolved in CH_2Cl_2. Reagents and CPG (1 μmol) were purchased from ABI and Glen Research. After preparation, the trityl-off oligonucleotide was cleaved off the resin and deprotected by treatment with conc. NH_4OH at r.t. for 24 hours. The oligonucleotide was dried and purified by HPLC on a RP-C18 column using the following conditions: A = NH_4OAc buffer (50 mM; pH 6.5); B = acetonitrile, flow rate 2.5 mL/min, UV detection at 260 nm (DNA) and at 548 nm (PBI). The oligonucleotides were lyophilizied and quantified by their absorbance at 528 nm in DMSO (ε = 62500 $M^{-1}cm^{-1}$) on a Varian Cary 100 spectrometer.

Modified oligonucleotides

Table S1 Sequences of MBs and target oligonucleotides

DNA	Sequence (3′→5′)
DNA1	A**PBI**CTAATTTGACCGTACGTCAGTTGACTGGTCAAATTA**PBI**CT
DNA2	A**PBI**CTAATGTACGTCAGTTGACTATTA**PBI**CT
DNA3	A**PBI**CAAGTACGTCAGTTGACTTT**PBI**CT
DNA4	A**PBI**CACGTACGTCAGTTGACTTT**PBI**CT
DNA5	ACTGGCATGCAGTCAACTGACCAG
DNA6	TAATAGTCAACTGACGTACATTA
DNA7	AAAGTCAACTGACGTACTT
DNA8	AAGTCAACTGACGTACG
DNA9	A**PBI**TAATCTTATAGTAGAAACCACAAAGTAATTA**PBI**CT
DNA10	TACTTTGTGGTTTCTACTATAAG

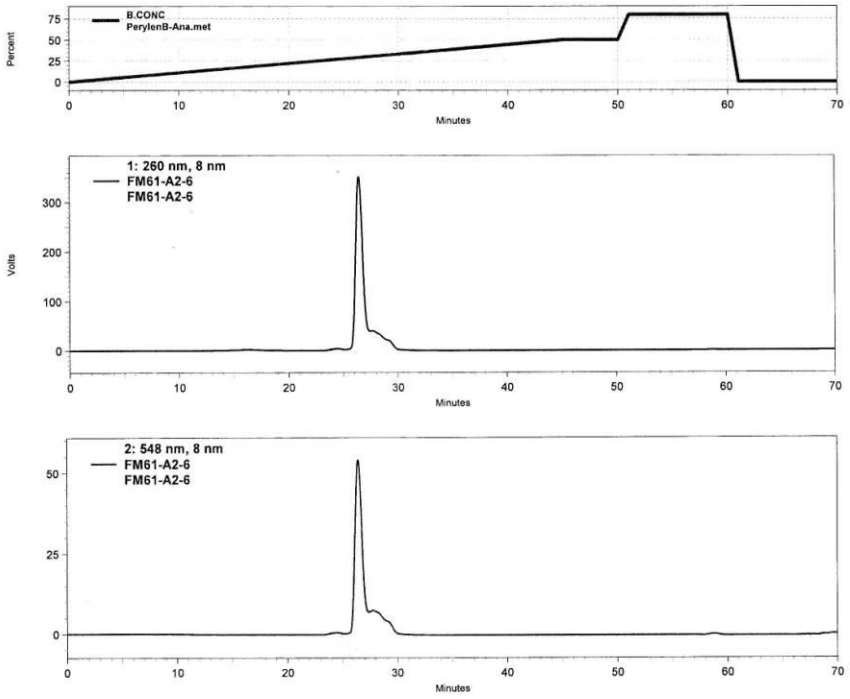
Table S2 Characterization of the PBI-modified oligonucleotides

DNA	$\varepsilon_{528\ nm}$ [mol L^{-1} cm^{-1}]	Mass calcd [Da]	Mass found [Da]
DNA 1	125 000	13226.4	1469.3 $[M-9H]^{9-}$ 1653.4 $[M-8H]^{8-}$ 1889.7 $[M-7H]^{7-}$ 2205.0 $[M-6H]^{6-}$
DNA 2	125 000	9520.8	1586.6 $[M-6H]^{6-}$ 1904.3 $[M-5H]^{5-}$ 2380.8 $[M-4H]^{4-}$
DNA 3	125 000	8286.6	1382.9 $[M+6H]^{6+}$ 1659.5 $[M+5H]^{5+}$ 2074.1 $[M+4H]^{4+}$
DNA4	125 000	8262.6	1377.0 $[M-6H]^{6-}$ 1652.6 $[M-5H]^{5-}$ 2066.0 $[M-4H]^{4-}$
DNA9	125 000	11985.3	1498.2 $[M-8H]^{8-}$ 1712.5 $[M-7H]^{7-}$ 1998.2 $[M-6H]^{6-}$ 2398.1 $[M-5H]^{5-}$

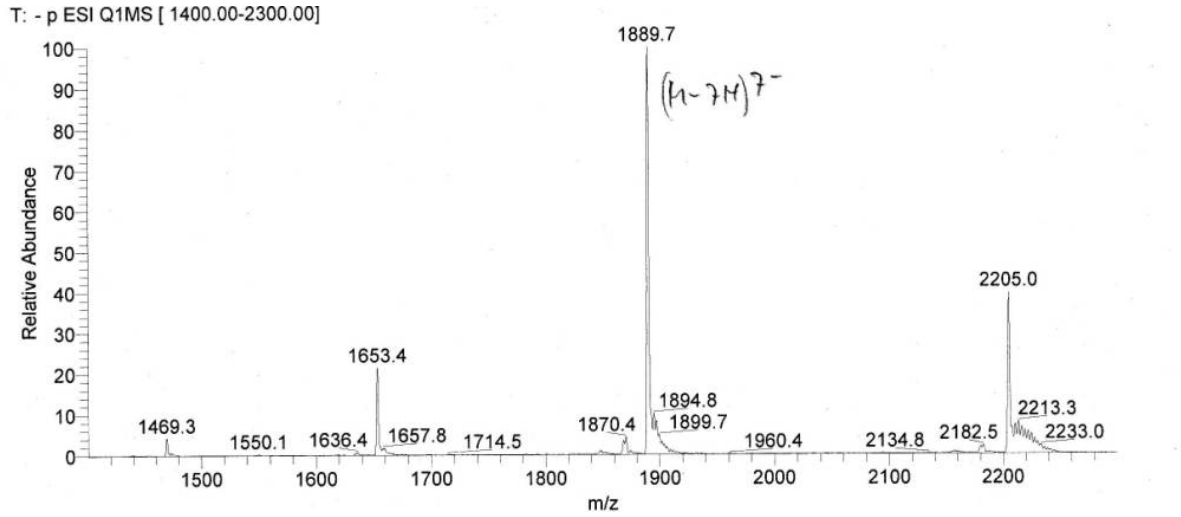
DNA1

3’-A**PBI**CTAATTTGACCGTACGTCAGTTGACTGGTCAAATTA**PBI**CT-5’

HPLC



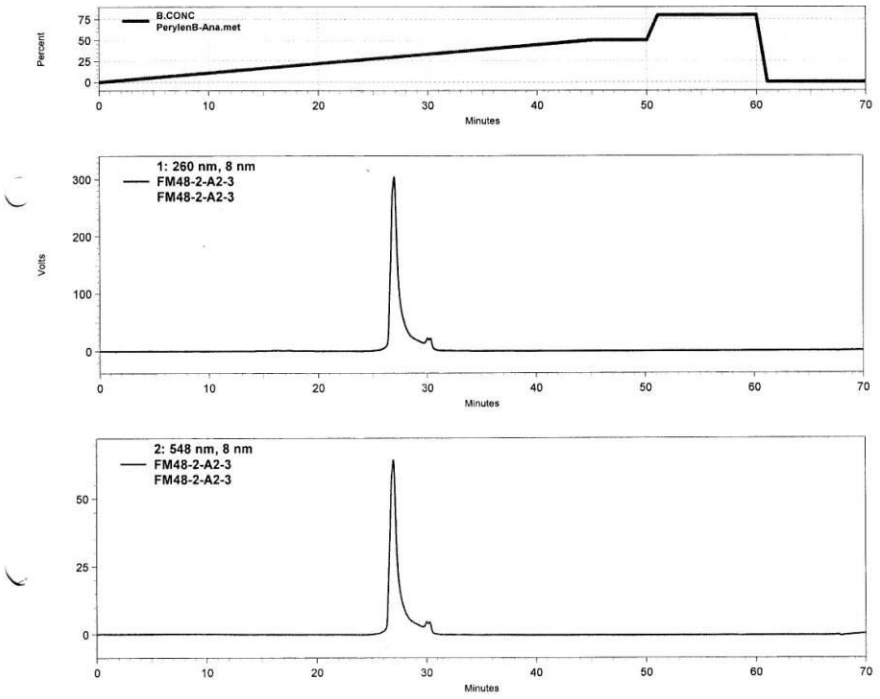
ESI-MS



DNA2

3’-A**PBI**CTAATGTACGTCAGTTGACTATTA**PBI**CT-5’

HPLC



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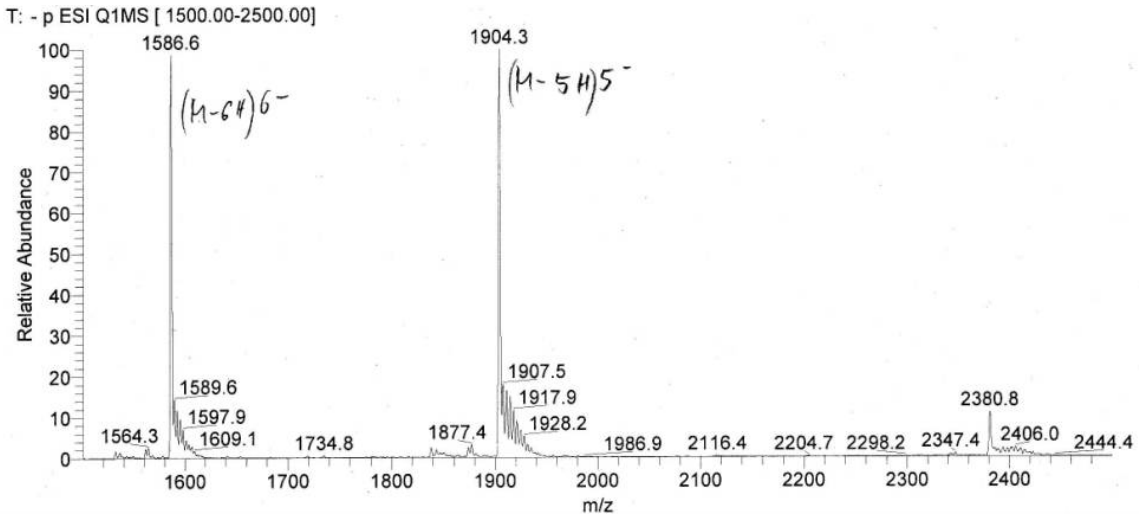
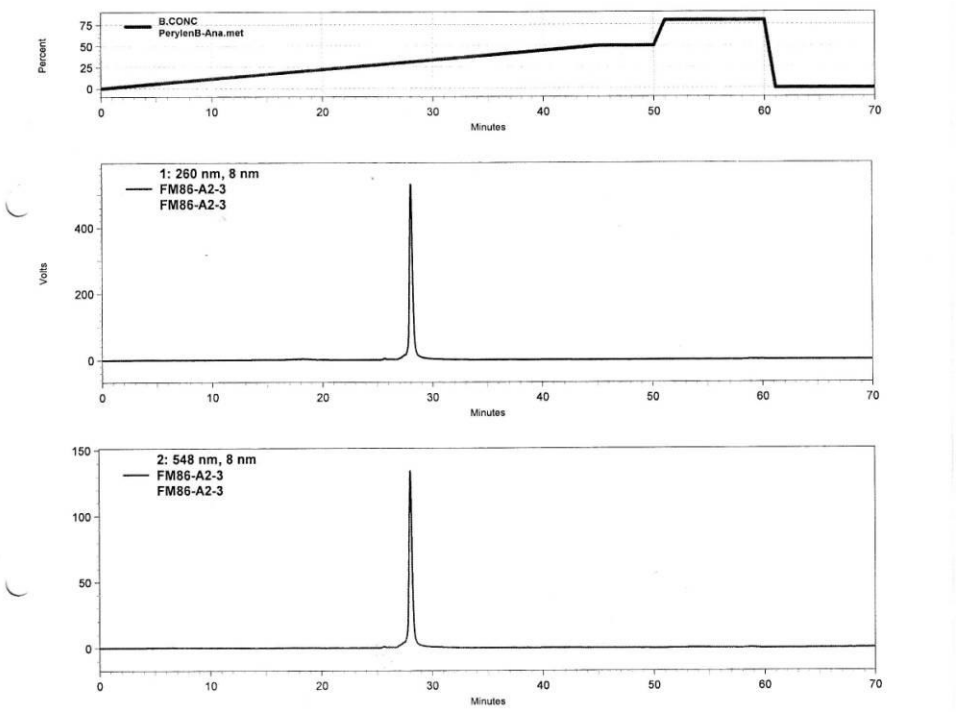


DNA3

3'-A**PBI**CAAGTACGTCAGTTGACTTT**PBI**CT-5'

HPLC



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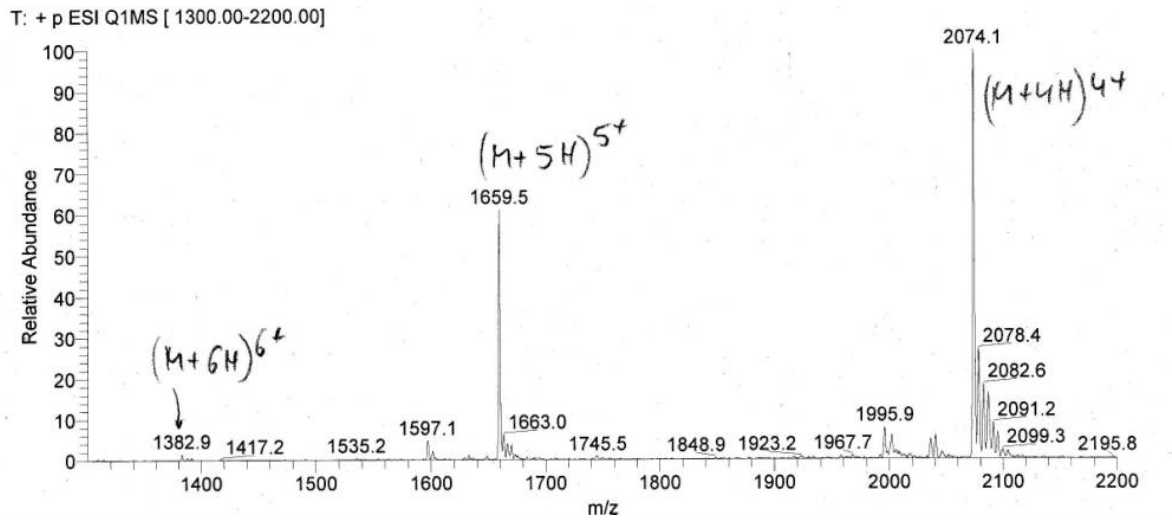
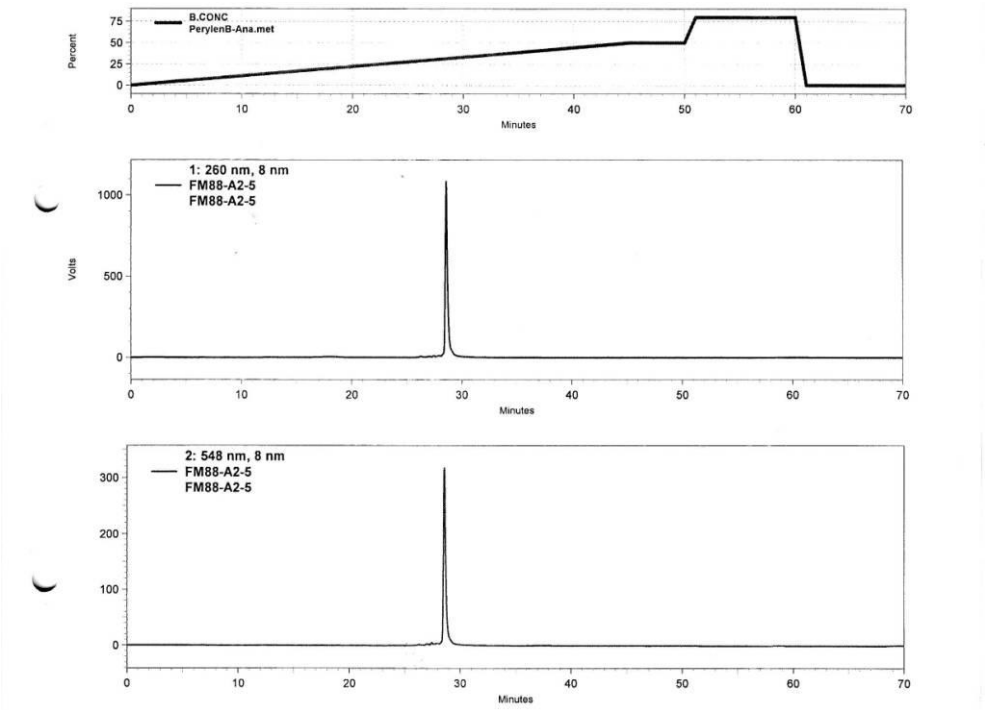


DNA4

3'-A**PBI**CACGTACGTCAGTTGACTTT**PBI**CT-5'

HPLC



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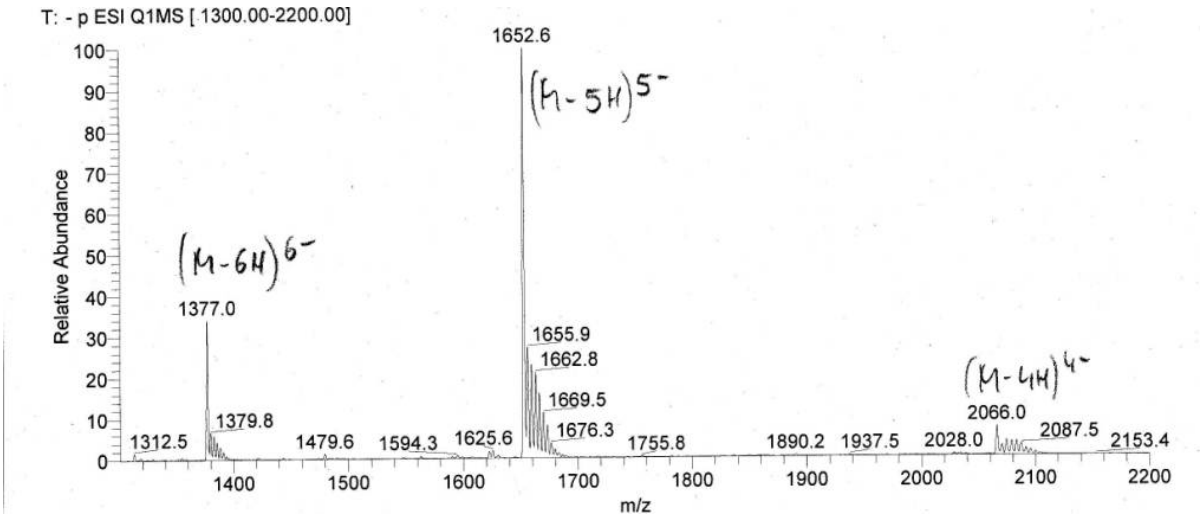
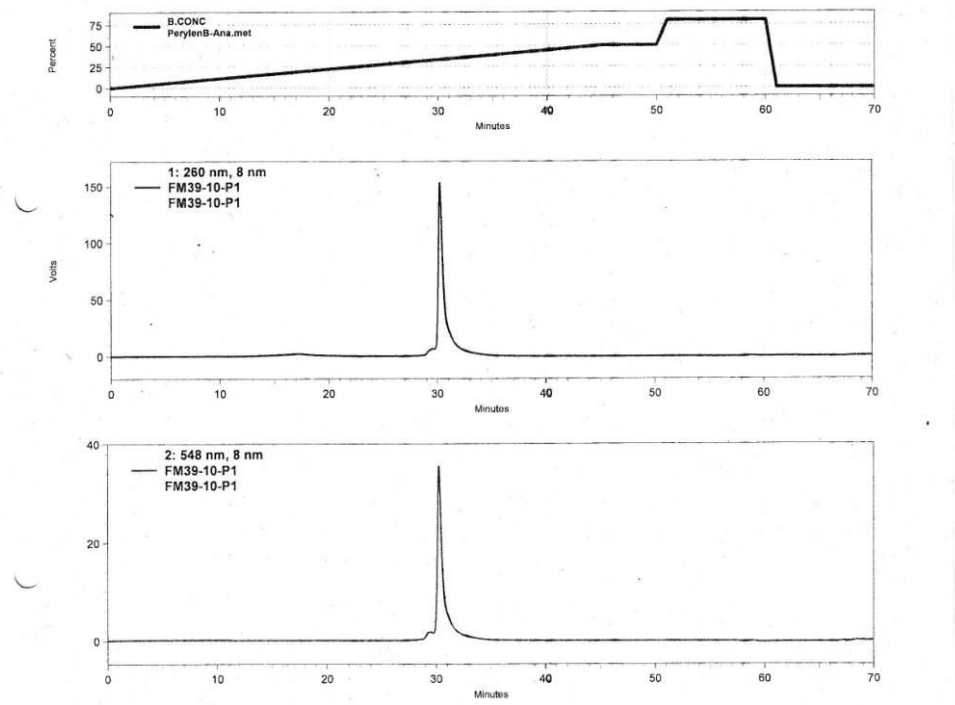


DNA9

3’-A**PBI**-TAA-TCT-TAT-AGT-AGA-AAC-CAC-AAA-GTA-ATT-A**PBI**C-T-5’

HPLC



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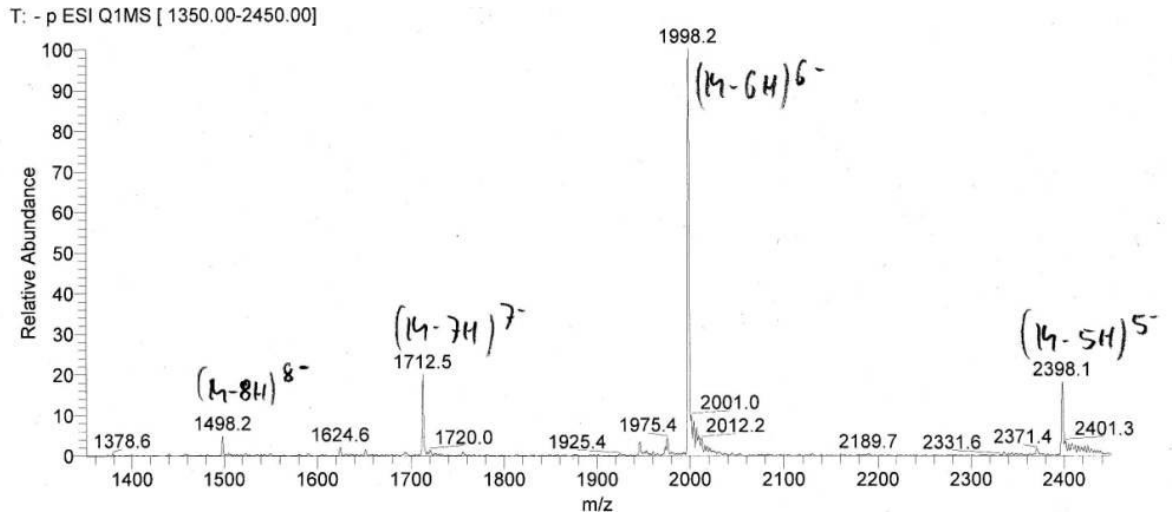

Titration Experiments

DNA3-7

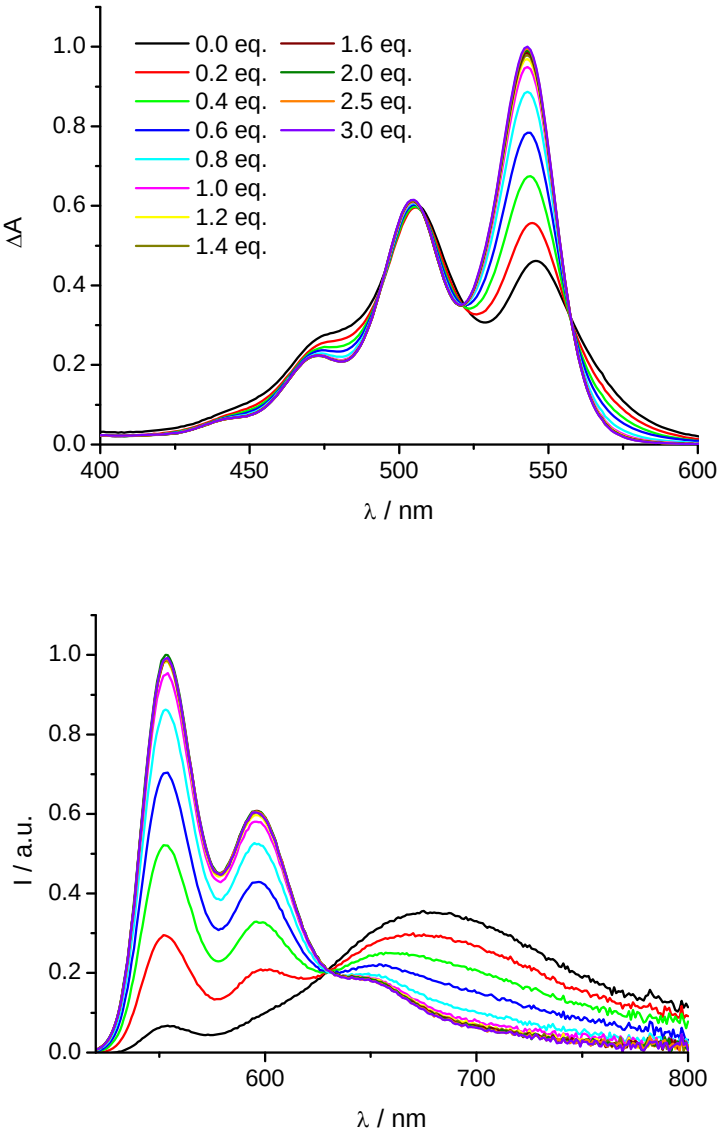

Fig. S1 Normalized UV/Vis absorption (top) and emission spectra of the titration of **DNA3** with **DNA7**, 2.5 μM DNA in 10 mM NaP_i-buffer, pH 7, 250 mM NaCl, λ_{exc} = 505 nm.

DNA4-8

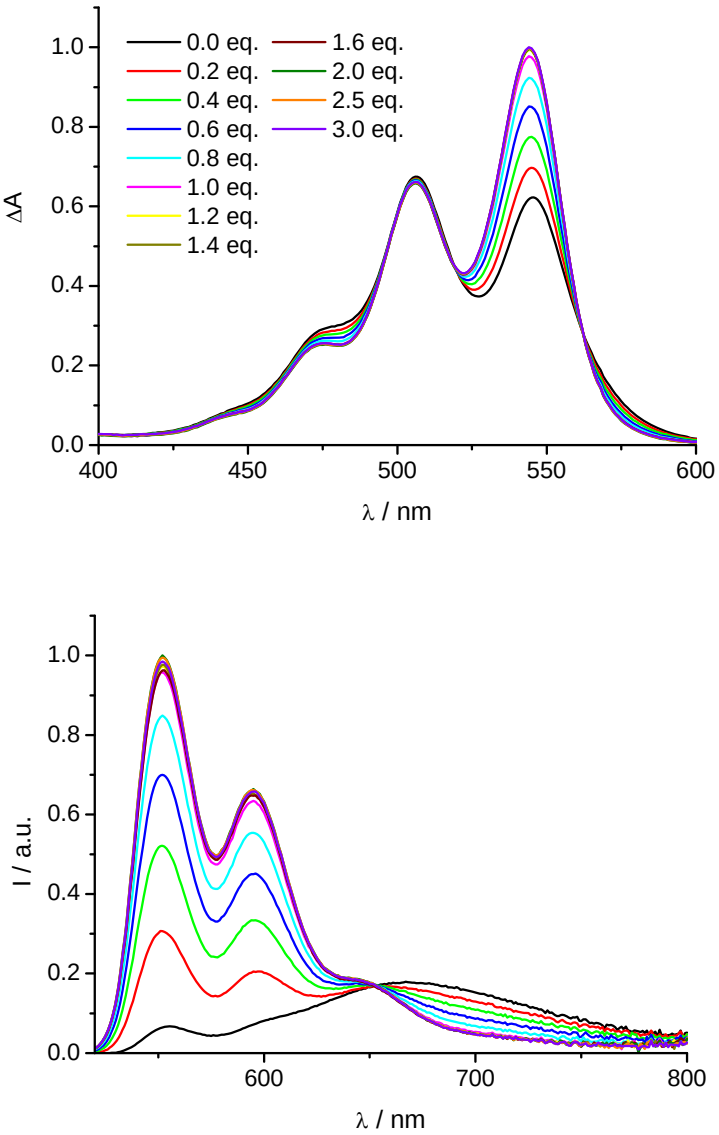

Fig. S2 Normalized UV/Vis absorption (top) and emission spectra of the titration of **DNA4** with **DNA8**, 2.5 µM DNA in 10 mM NaP_i-buffer, pH 7, 250 mM NaCl, λ_{exc} = 505 nm.

DNA9-10

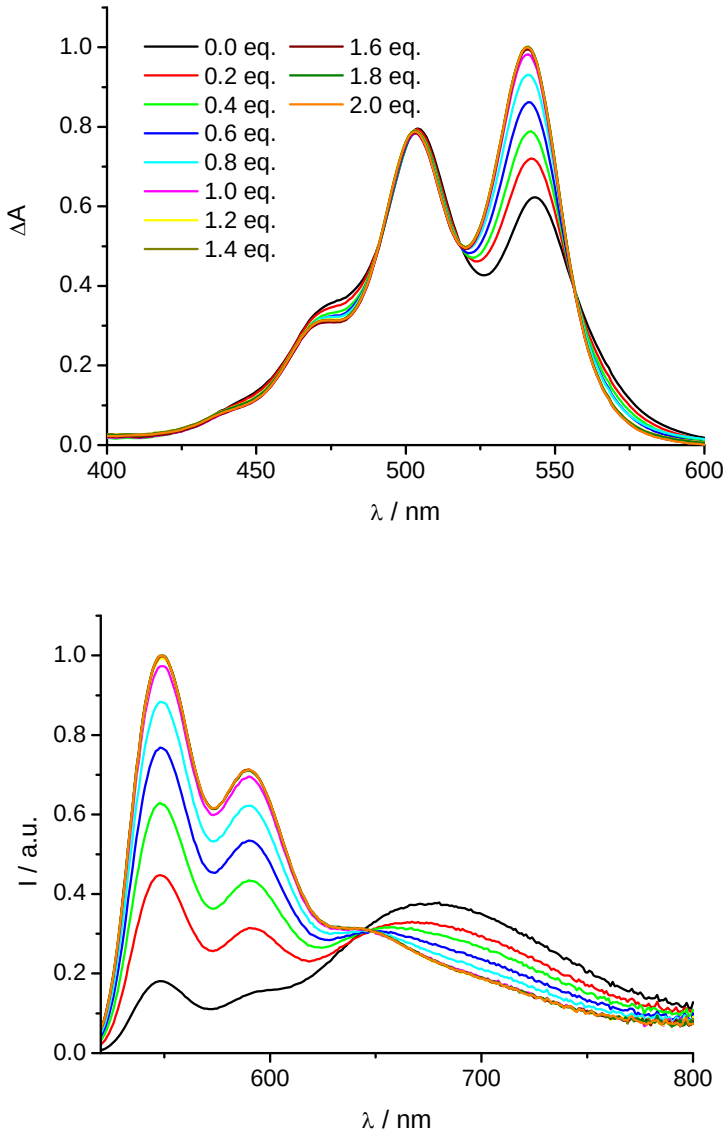

Fig. S3 Normalized UV/Vis absorption (top) and emission spectra of the titration of **DNA9** with **DNA10**, 2.5 µM DNA in 10 mM NaP_i-buffer + 40 vol-% EtOH, pH 7, 250 mM NaCl, λ_{exc} = 505 nm**.**

DNA2-6 + 40 vol-% EtOH

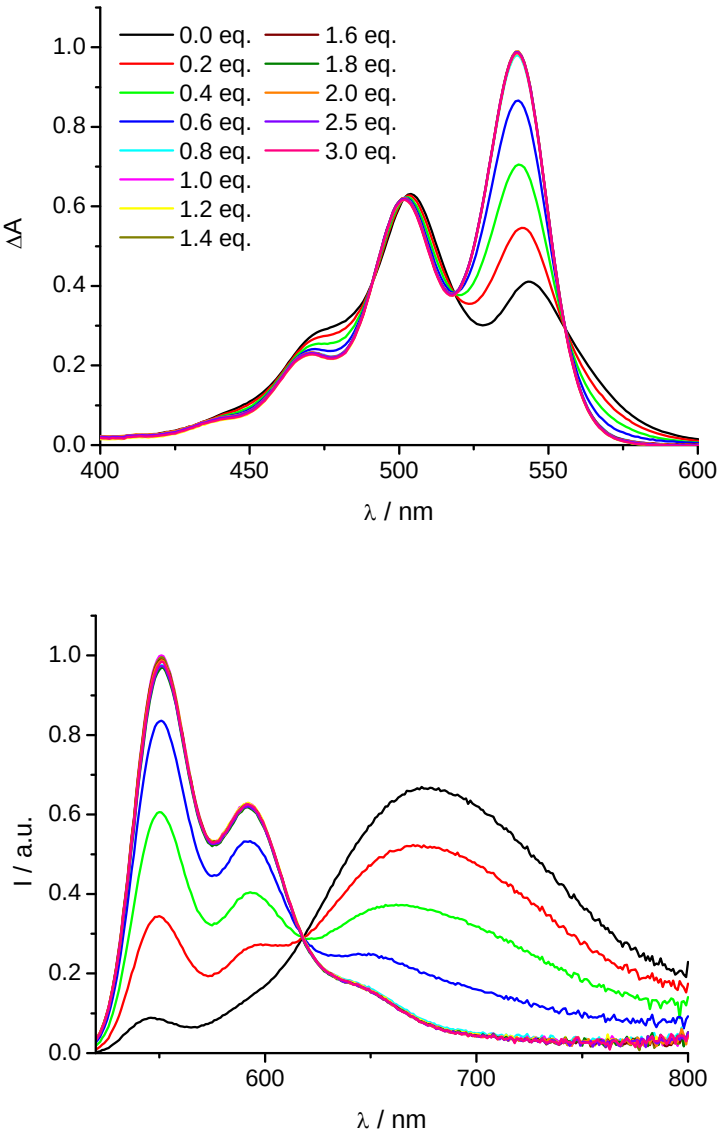

Fig. S4 Normalized UV/Vis absorption (top) and emission spectra of the titration of **DNA2** with **DNA6**, 2.5 µM DNA in 10 mM NaP_i-buffer + 40 vol-% EtOH, pH 7, 250 mM NaCl, λ_{exc} = 505 nm.

DNA9-10 + 40 vol-% EtOH

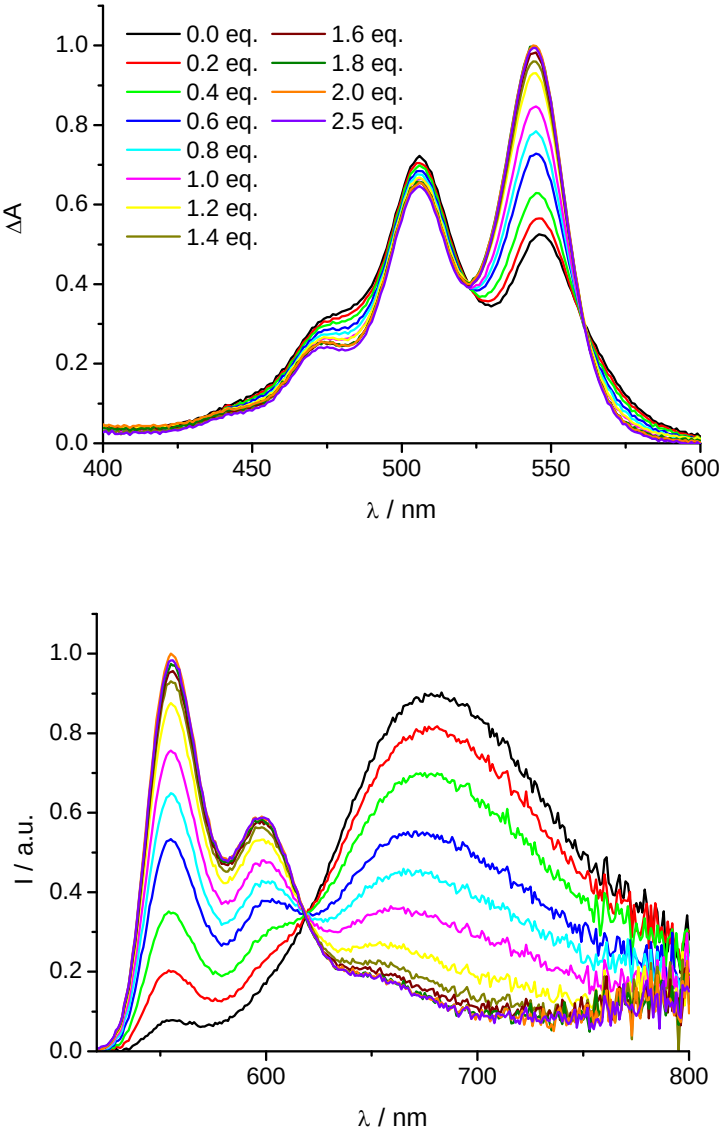

Fig. S5 Normalized UV/Vis absorption (top) and emission spectra of the titration of **DNA9** with **DNA10**, 2.5 µM DNA in 10 mM NaP_i-buffer + 40 vol-% EtOH, pH 7, 250 mM NaCl, λ_{exc} = 505 nm.

Table S1 Melting temperatures (T_m) of the hairpin **DNA9** and the corresponding full duplex.[a]

hairpin	T_m (°C)	full duplex	T_m (°C)	ΔT_m (°C)
DNA9	46.2	**DNA9-10**	64.5	+18.3
DNA9[b]	23.7	**DNA9-10**[b]	42.8	+19.1

[a] λ = 260 nm, 20-90 °C, interval: 0.7 °C/min, 2.5 µM DNA in 10 mM NaP_i-buffer (pH 7.0), 250 mM NaCl. [b]In the presence of 40 vol-% ethanol in the buffer solution.

Kinetics of hairpin opening

DNA2 in NaP_i-buffer

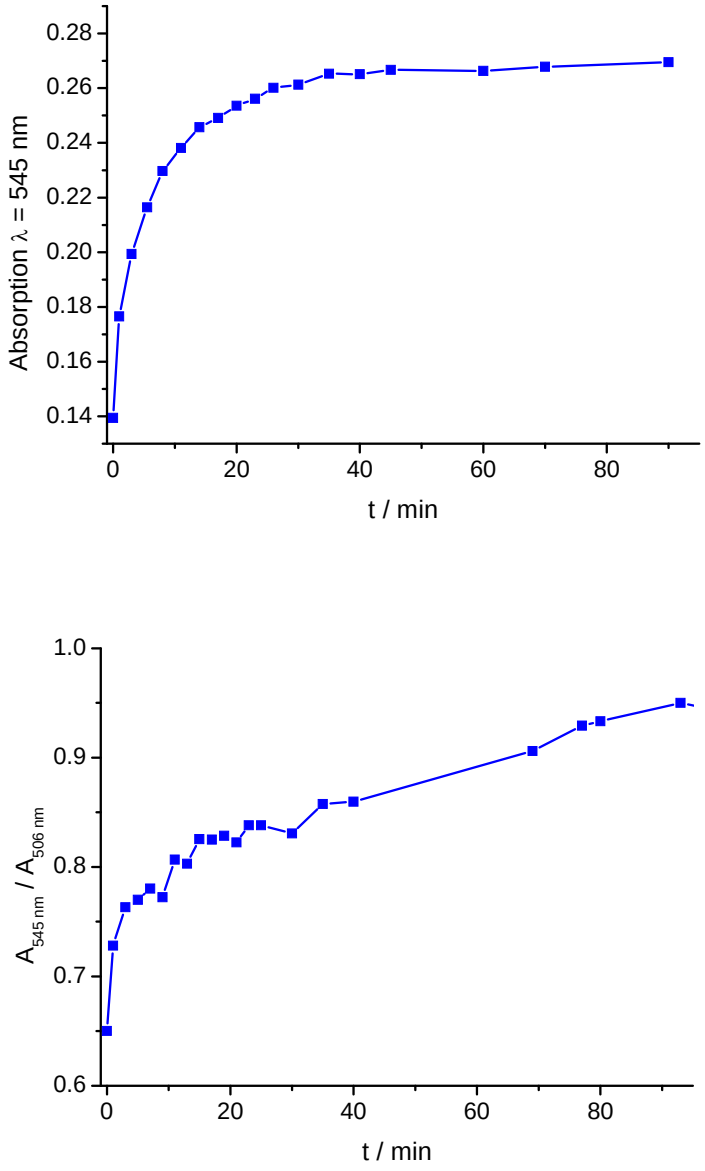

Fig. S6 Time dependent observation of the absorption (λ = 545 nm; top) and the absorption ratio (A545 / A506; bottom) of **DNA2** after addition of 0.5 eq. of **DNA5**, 2.5 µM DNA in 10 mM NaP_i-buffer, pH 7, 250 mM NaCl.

DNA2 and DNA9 in NaPi-buffer + 40 vol-% EtOH

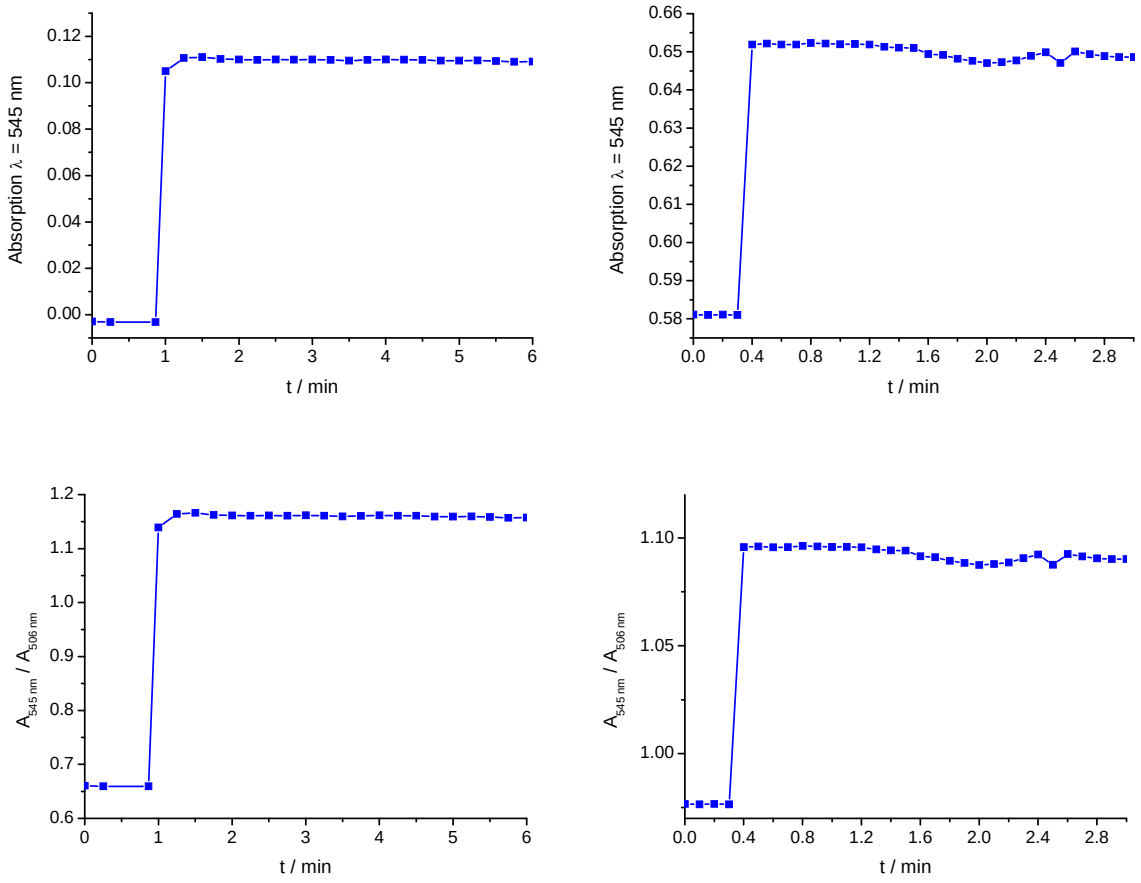

Fig. S7: Time dependent observation of the absorption (λ = 545 nm; top line) and the absorption ratio (A545 / A506; bottom line) of **DNA2** after addition of 0.5 eq. of **DNA5** (top left), and **DNA9** (top right) after addition of 0.5 eq. of **DNA10** (right), 2.5 µM DNA in 10 mM NaP_i-buffer + 40 vol-% EtOH, pH 7, 250 mM NaCl.