Supplementary Information

Strand Displacement and Duplex Invasion into Double-Stranded DNA by Pyrrolidinyl Peptide Nucleic Acids

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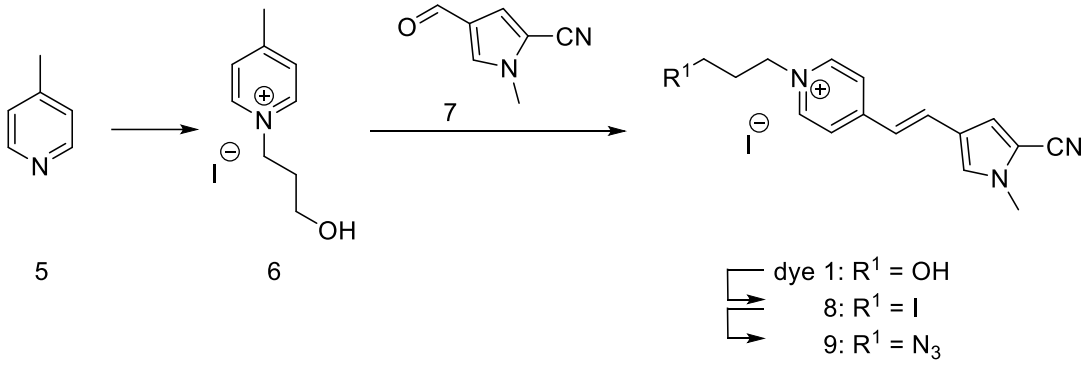
2. Materials and methods:

Chemicals and dry solvents were purchased from *Aldrich*, *ABCR* and *VWR* were used without further purification unless otherwise mentioned. Unmodified DNA strands were obtained from *Metabion*. TLC was performed on ALUGRAM Sil G/UV_{254} 0,20 nm silica gel 60 F254 from *Macherey-Nagel GmbH & Co. KG*. Flash chromatography was carried out with silica gel 60 from Aldrich (60 – 43 µm). Spectroscopic measurements were recorded in NaP_i buffer solution (10 mM, pH = 7) in presence or absence of 250 mM NaCl (see detailed description for each experiment) using quartz glass cuvettes (10 mm). Absorption spectra were recorded with a *Varian* Cary 100 spectrometer equipped with a 6x6 cell changer unit at 20 °C. 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 at 20 °C and are corrected for Raman emission from the buffer solution. Fluorescence lifetimes were measured with Horiba Scientific FluoroMax-4 spectrofluorometer using a time-correlated single photon counting (TCSPC) technique with excitation sources NanoLed at 370 nm or 455 nm (Horiba, impulse repetition rate of 1 MHz, time calibration = 2.74E-11 sec/ch). Lifetimes were calculated with DAS6 v 6.8 decay analysis software (Horiba). The determination of FAB mass spectra was executed by the Institute of Organic Chemistry of the KIT using a *Finnigan* MAT95 in positive ionization mode. NMR spectra were recorded on a *Bruker* B-ACS-60, *Bruker* Avance DRX 400 and a *Bruker* Avance DRX 500 spectrometer in deuterated solvents (^{1}H at 300, 400 or 500 MHz, ^{13}C at 75, 100 or 125 MHz). Chemical shifts are given in ppm relative to TMS. IR spectra recording were performed by the Institute of Organic Chemistry of the KIT with a *Bruker* IFS88. DNA strands were purified with a Reversed Phase *Supelcosil™* LC-C18 column (250 x 10 mm, 5 µm) on a *Shimadzu* HPLC system (autosampler SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A). Purification was verified by MS (MALDI) on a Biflex-IV spectrometer from Bruker Daltonics in the linear negative mode and Autoflex-III Smartbeam from Bruker Daltonics in the linear negative mode (matrix for DNA: 2:1 mixture of 2,4,6-trihydroxyacetophenone (0.3 M in EtOH) and diammoniumcitrate (0.1 M in H_2O); matrix for PNA: saturated α-cyano-4-hydroxycinnamic acid (CCA) solution with H_2O:acetonitrile (1:1) + 0.1 % TFA). Finally the oligonucleotides were lyophilized and quantified by their absorbance in water at 260 nm on a ND-1000 spectrophotometer from NanoDrop in the nucleic acid mode.

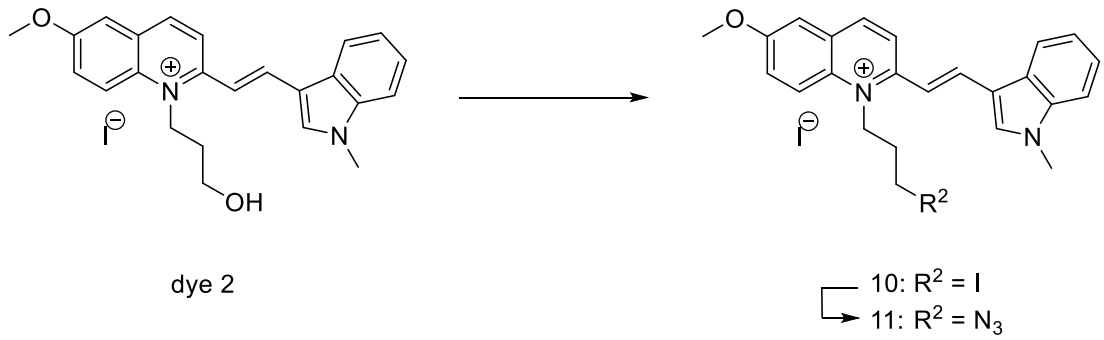
3. Schematic synthesis of the dyes 1-4:

3.1 Dye 1 and corresponding azide 8:



Scheme S01: Synthesis of dye 1 and corresponding azide 9.

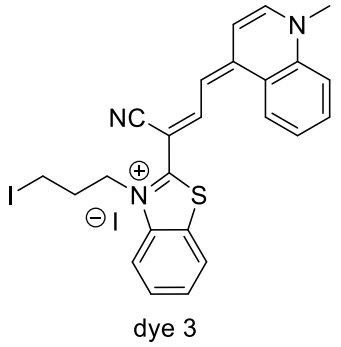
3.2 Dye 2 and corresponding azide 11:



Scheme S02: Dye 2 and corresponding azide 11.

Synthesis of dye 2 is already published.[1]

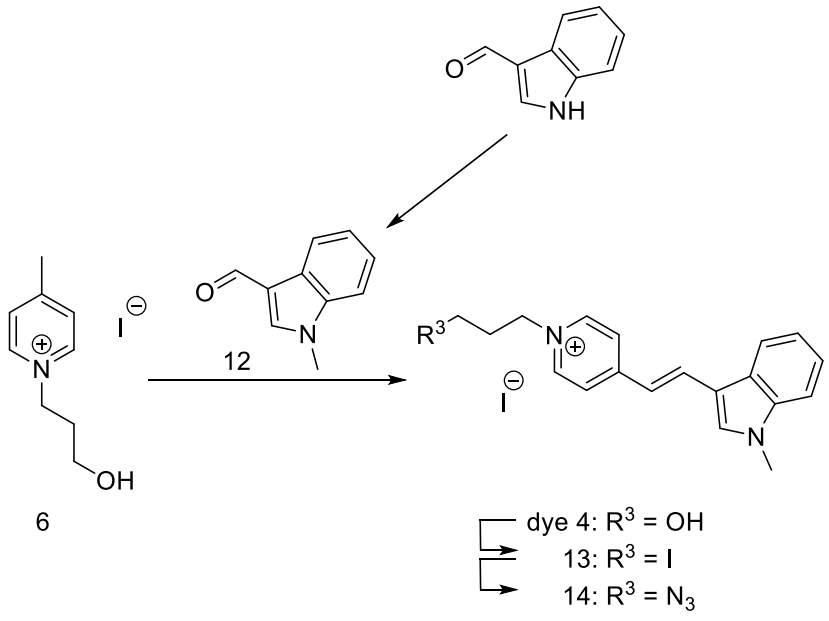
3.3 Dye 3 and corresponding azide:



Scheme S03: Dye 3.

Synthesis of dye 3 and the corresponding azide is already published.[2]

3.4 Dye 4 and corresponding azide 14:

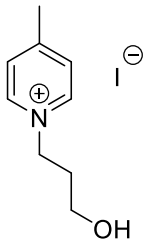


Scheme S04: Synthesis of dye 4 and corresponding azide 14.

4. Synthesis:

4.1 Synthesis of compound 6:

1-(3-hydroxypropyl)-4-methylpyridin-1-ium iodide



Under argon, a mixture of 4-methylpyridine (**5**, 0.47 g, 0.49 mL, 5.0 mmol) and 3-iodo-1-propanol* (0.72 mL, 1.40 g, 7.5 mmol) in 3 mL 1,4-dioxane was stirred in a headspace vial at 101°C for 16 h. After cooling to room temperature 7 mL diethyl ether were added and mixed thoroughly. Then the upper layer was removed and the procedure repeated for 2 times. Drying under reduced pressure yields brown oil (quant.).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

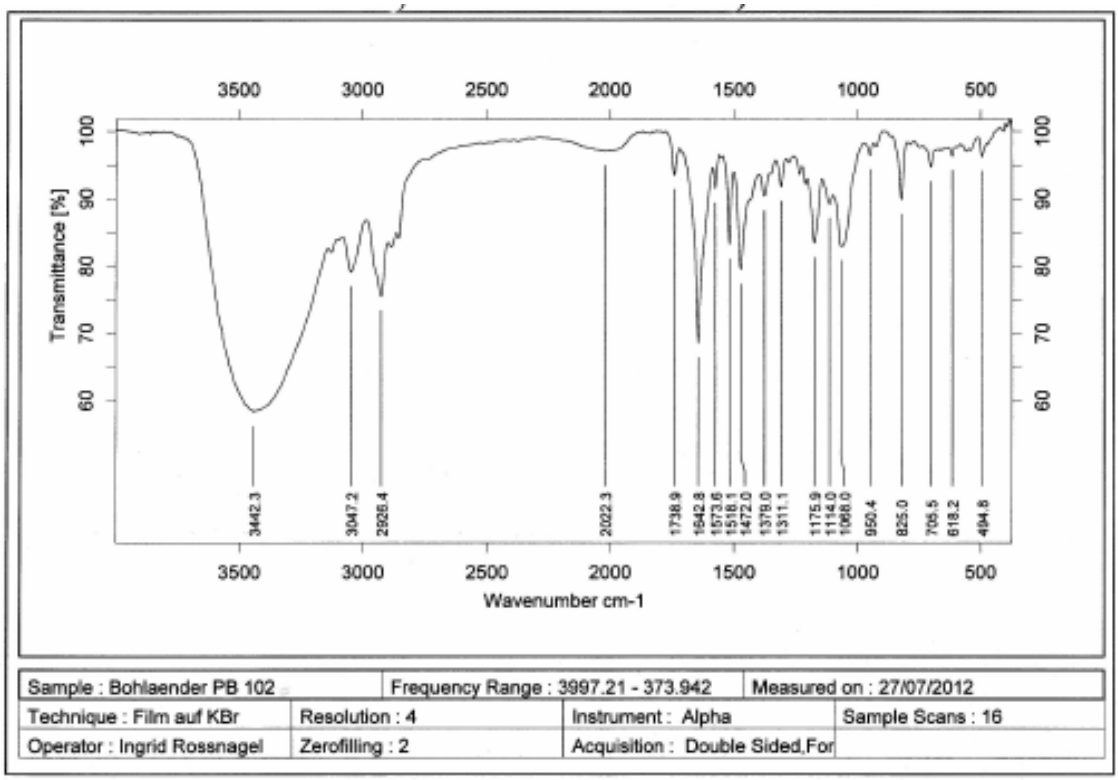
TLC (dichloromethane : methanol = 9 : 1): R_f = 0.04.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3442 (s), 3047 (m), 2926 (m), 1643 (m), 1068 (w).

^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 1.97 – 2.12 (m, 2H), 2.60 (s, 3H),), 3.40 – 3.45 (m, 2H), 3.45 – 3.84 (m, 1H), 4.61 (t, J = 7.0, 2H), 7.98 (d, J = 6.1, 2H), 8.88 – 8.97 (m, 2H).

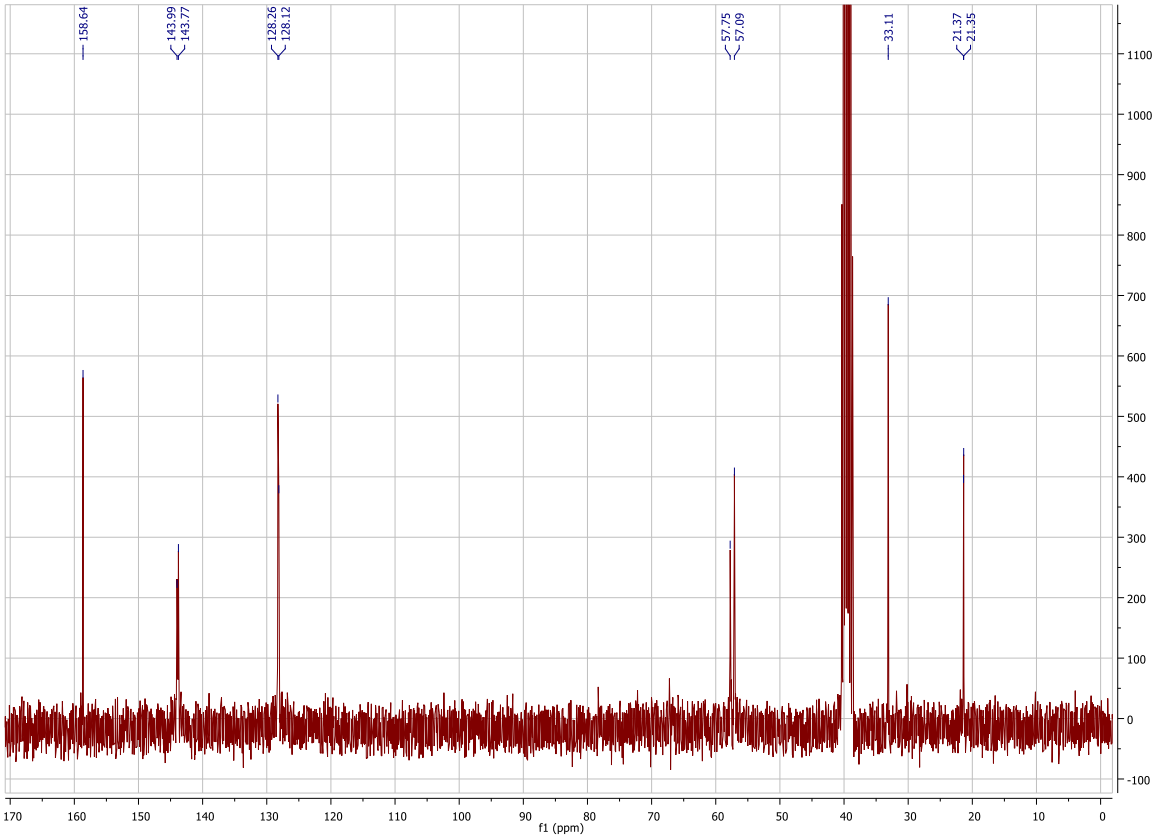
^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 21.4, 33.1, 57.1, 57.8, 128.1, 128.3, 143.8, 144.0, 158.6.

MS (FAB) m/z (%): 152.6 (100) [M^+].

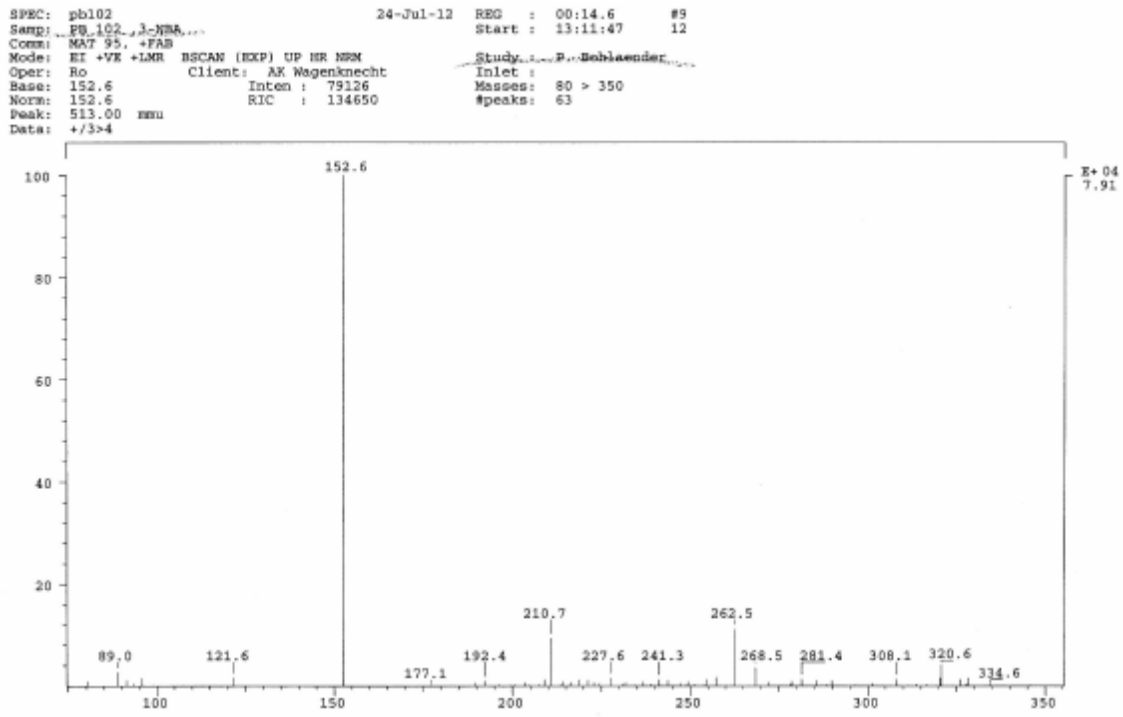


Scheme S05: IR of compound **6**.

Scheme S06: ^{1}H-NMR of compound **6**.



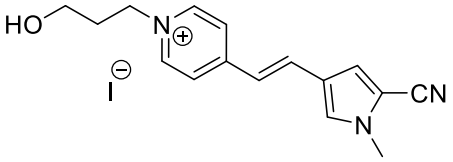
Scheme S07: ^{13}C-NMR of compound **6**.



Scheme S08: MS (FAB) of compound **6**.

4.2 Synthesis of dye 1:

(E)-4-(2-(5-cyano-1-methyl-1H-pyrrol-3-yl)vinyl)-1-(3-hydroxypropyl)pyridin-1-ium iodide



Under argon, to a mixture of compound **6** (0.21 g, 1.00 mmol) and 4-Formyl-1-methyl-1H-pyrrol-2-carbonitril **7** (0.20 g, 2.00 mmol) in 10 mL ethanol, piperidine (0.14 g, 0.16 mL, 1.65 mmol) was added and the reaction mixture was stirred in a headspace vial at 65°C for 19 h. After cooling to room temperature the precipitated product was collected and washed three times with diethyl ether. Drying under reduced pressure yields a light-brown solid (69 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.10.

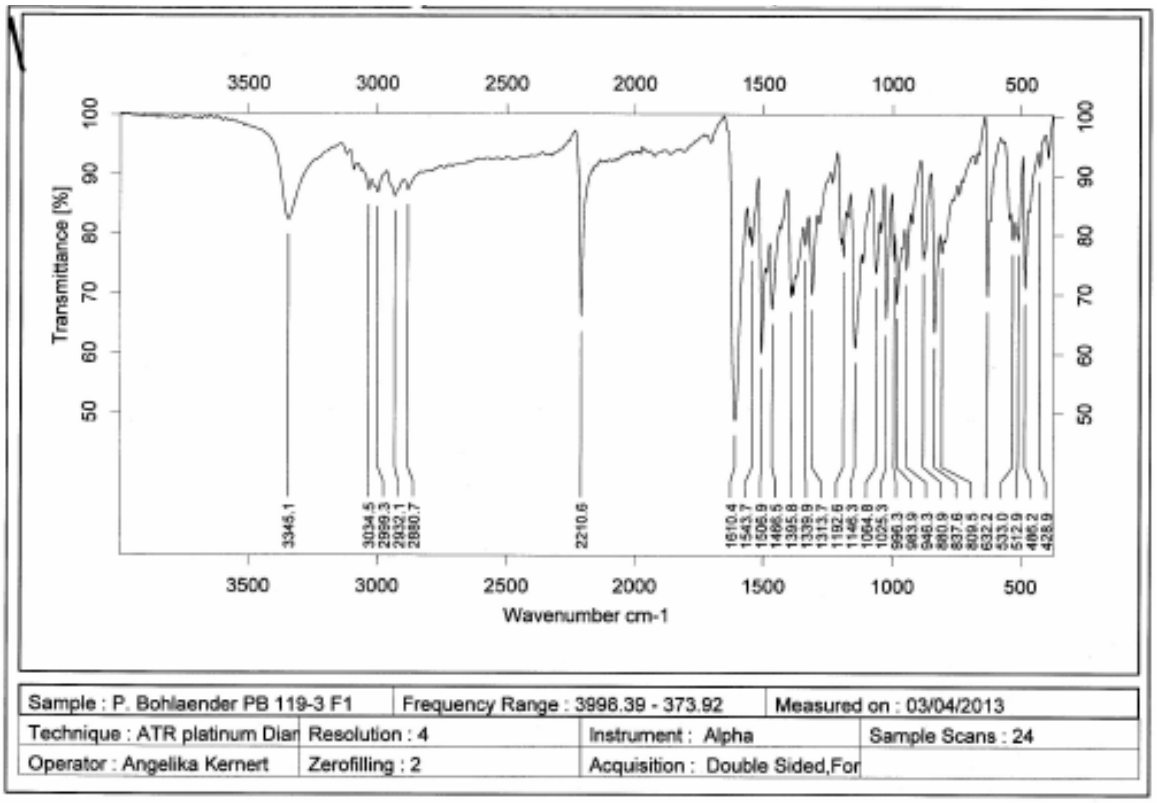
IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3345 (s), 2211 (s), 1610 (s), 1314 (w), 1146 (m).

^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 1.93 - 2.13 (m, 2H), 3.39 - 3.53 (m, 2H), 3.81 (s, 3H), 4.44 - 4.63 (m, 2H), 4.74 (s, 1H), 7.13 (d, *J* = 16.0, 1H), 7.41 (s, 1H), 7.64 (s, 1H), 7.87 (d, *J* = 15.8, 1H), 8.08 (d, *J* = 5.6, 2H), 8.84 (d, *J* = 5.9, 2H).

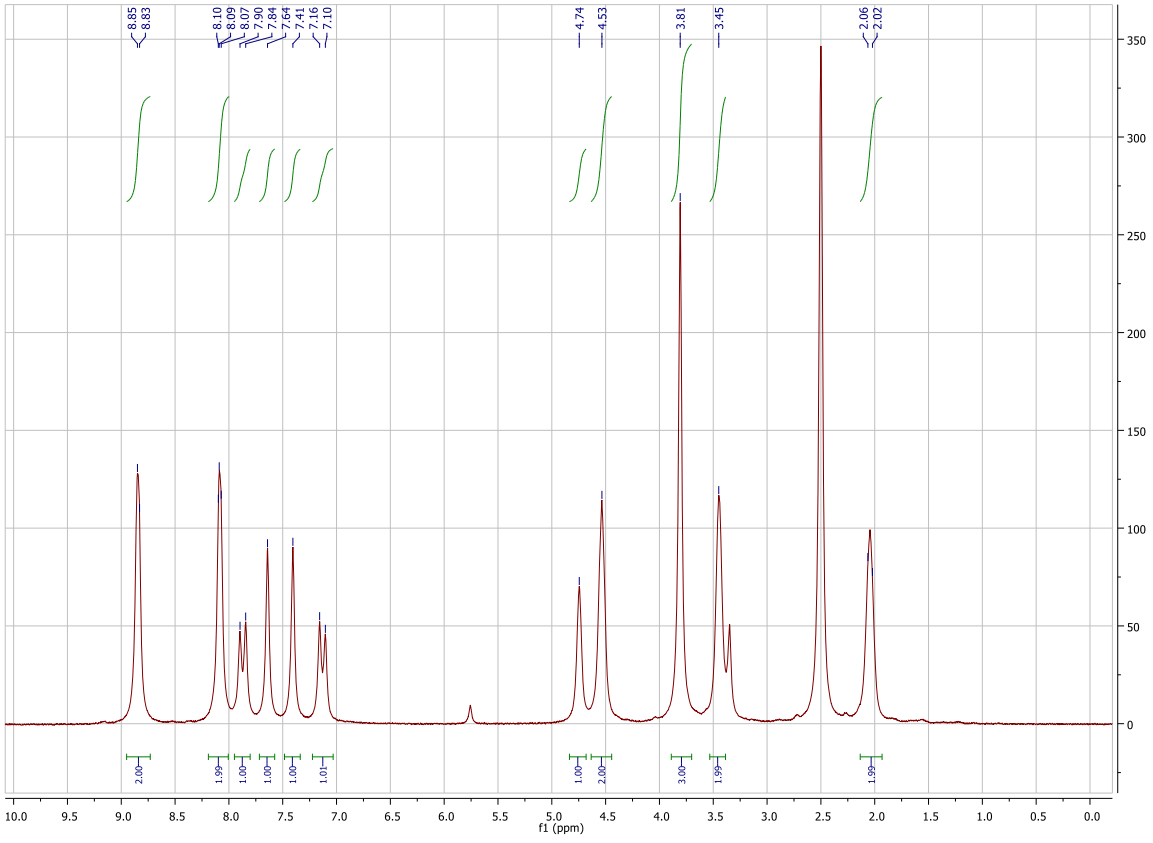
^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 33.1, 35.5, 57.1, 57.3, 105.6, 113.0, 117.9, 120.5, 121.3, 122.8, 131.0, 133.8, 144.2, 153.2.

MS (FAB) m/z (%): 268.1 (100) [M$^+$].
HR-MS (FAB) m/z: calculated for $C_{16}H_{18}N_3O$ [M$^+$]: 268.1450, found: 268.1448.

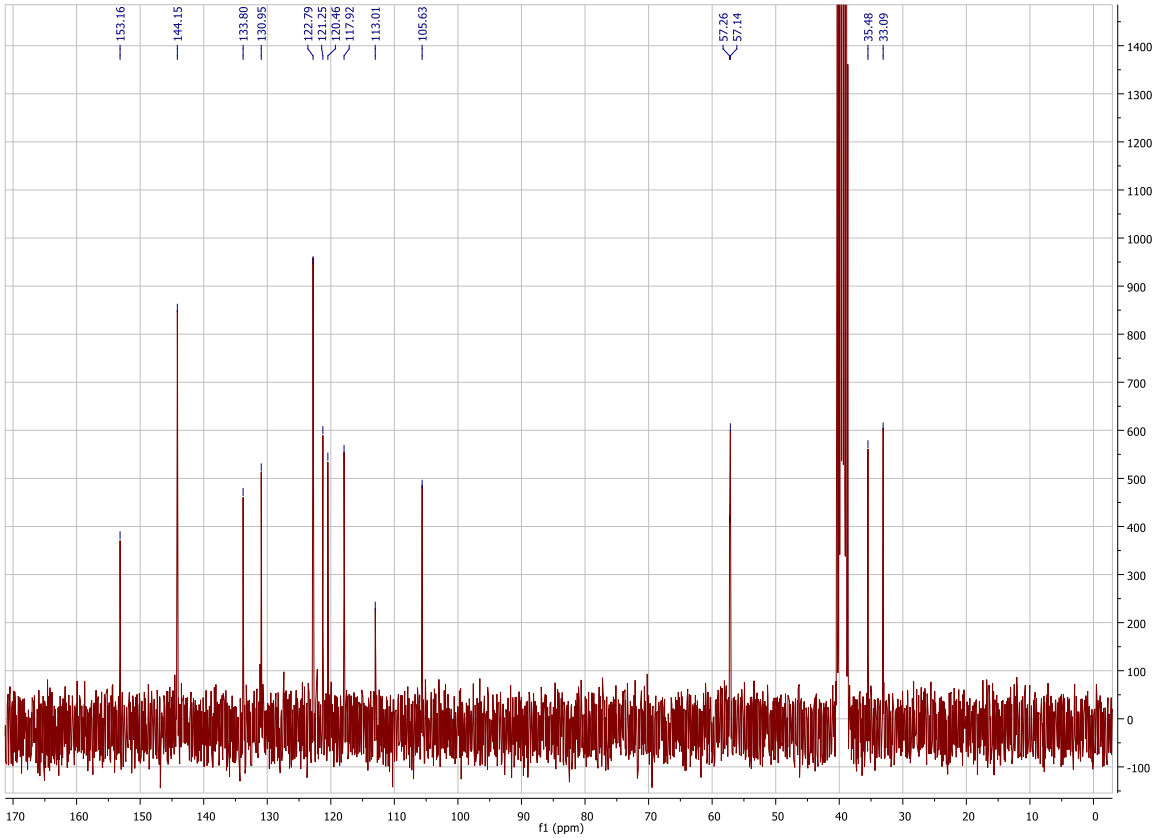
Elementary analysis
calculated for $C_{16}H_{18}IN_3O$:
N: 10.63 % → found: 10.45 % C: 48.62 % → found: 48.71 %
H: 4.59 % → found: 4.39 %



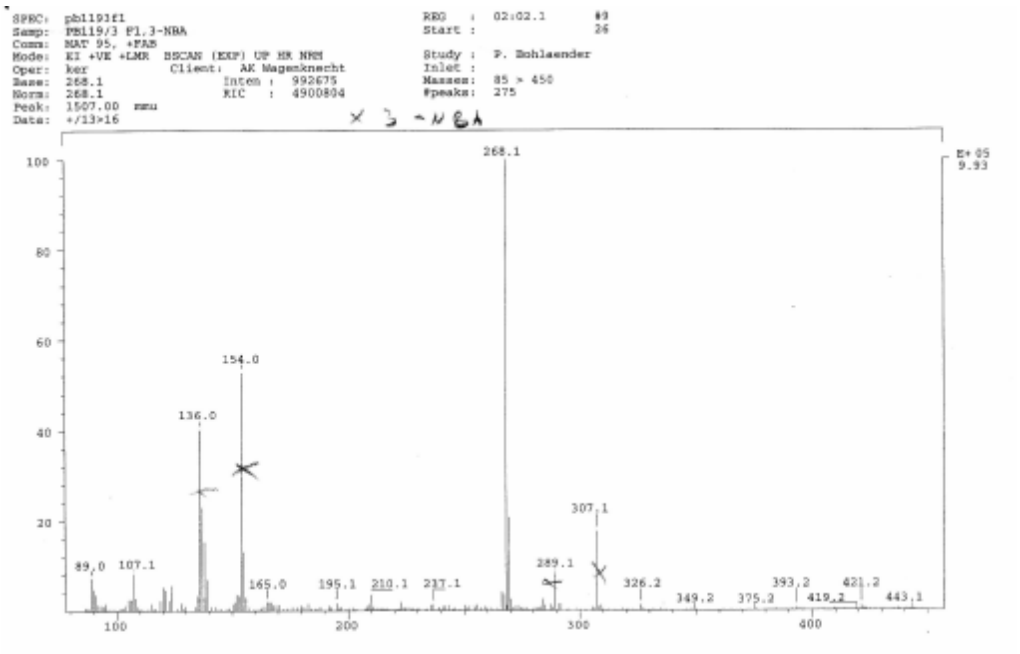
Scheme S09: IR of dye **1**.



Scheme S10: ^{1}H-NMR of dye **1**.



Scheme S11: ^{13}C-NMR of dye **1**.



Scheme S12: MS (FAB) of dye **1**.

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LIST:  pb1193f1-c1                          02-Apr-13   Elapse:   02:25.0      19
Samp:  PB119/3 F1,3-NBA                                 Start :   13:26:09     48
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM               Study :   P. Bohlaender
Oper:  ker            Client:  AK Wagenknecht           Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 268 ) C16.H18.O.N3
Peak:  1507.00  mmu    R+D:  -0.5 > 65.0
Data:  CMASS : converted

            310457                    (mmu)
   Mass  Intensity     %RA   Flags Delta  R+D  Composition
268.1448    1034859  100.00   F#      0.2  9.5  C16.H18.O.N3
```

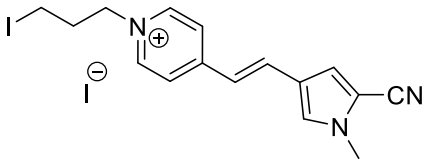
Scheme S13: HR-MS (FAB) of dye **1**.

Berechnet:	N: C: H: S:
Gefunden:	N: C: H: S:
Gefunden:	N: C: H: S:

Scheme S14: Elementary analysis of dye **1**.

4.3 Synthesis of compound 8:

(E)-4-(2-(5-cyano-1-methyl-1H-pyrrol-3-yl)vinyl)-1-(3-iodopropyl)pyridin-1-ium iodide



Under argon, a mixture of dye **1** (0.20 g, 0.50 mmol), triphenylphosphine (0.39 g, 1.50 mmol) and tetrabromomethane (0.55 g, 1.65 mmol) in 5 mL dichloromethane was stirred in a headspace vial at room temperature for 2 h. After addition of 2 g NaI to the mixture it was solubilized with 75 mL acetone and 10 mL methanol and the solvent was removed at 50°C and reduced pressure. The residue was dissolved in 20 mL acetone and 8 mL methanol and stirred at 50°C for 90 h. After cooling to room the precipitation was removed and the filtrate was reduced to a residual volume of 5 mL. The suspension was diluted with 5 mL methanol and the product was crystallized with use of ultra sonic bath. The precipitation of the crude product was collected and washed three times with

diethylether and solubilized in 150 mL dichloromethane and 50 mL water. The aqueous phase was extracted additional two times with 50 mL dichloromethane, respectively. The solvent was removed at 40°C and reduced pressure. The residue was suspended in 10 mL methanol using ultra sonic bath. The precipitation of the product was collected and washed three times with diethylether. Drying under reduced pressure yields light-brown solid (95 %).

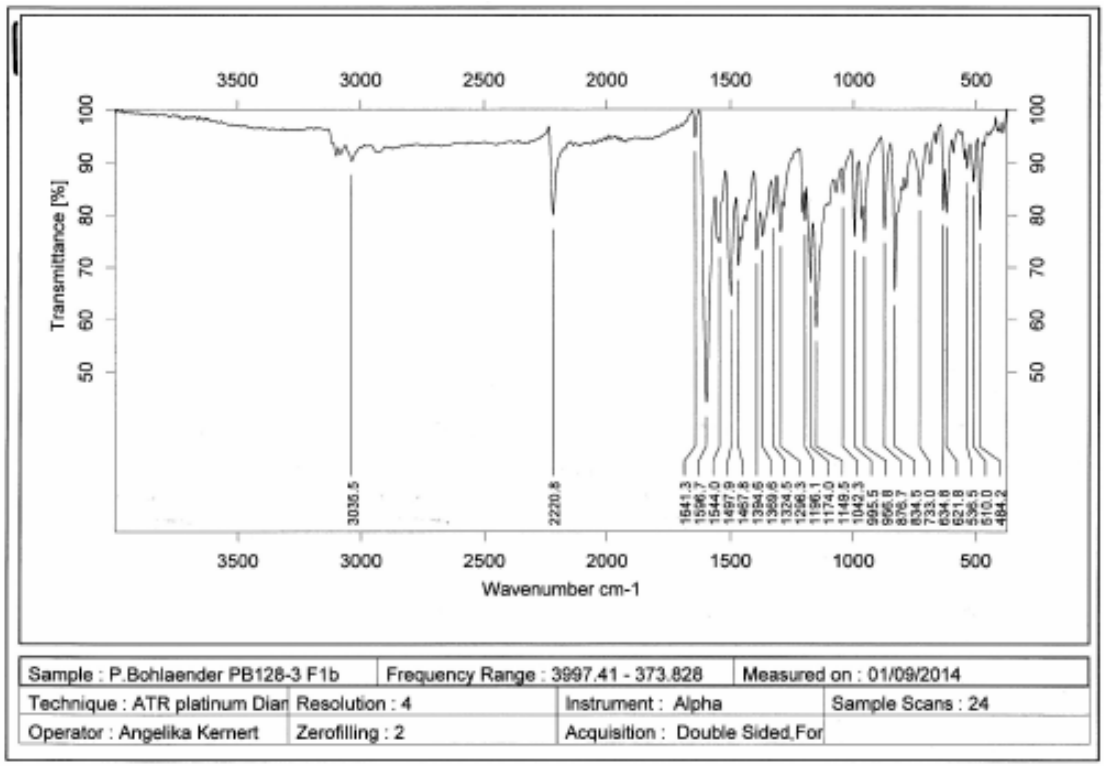
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.18.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3036 (w), 2221 (s), 1597 (s), 1501 (m), 1150 (m).

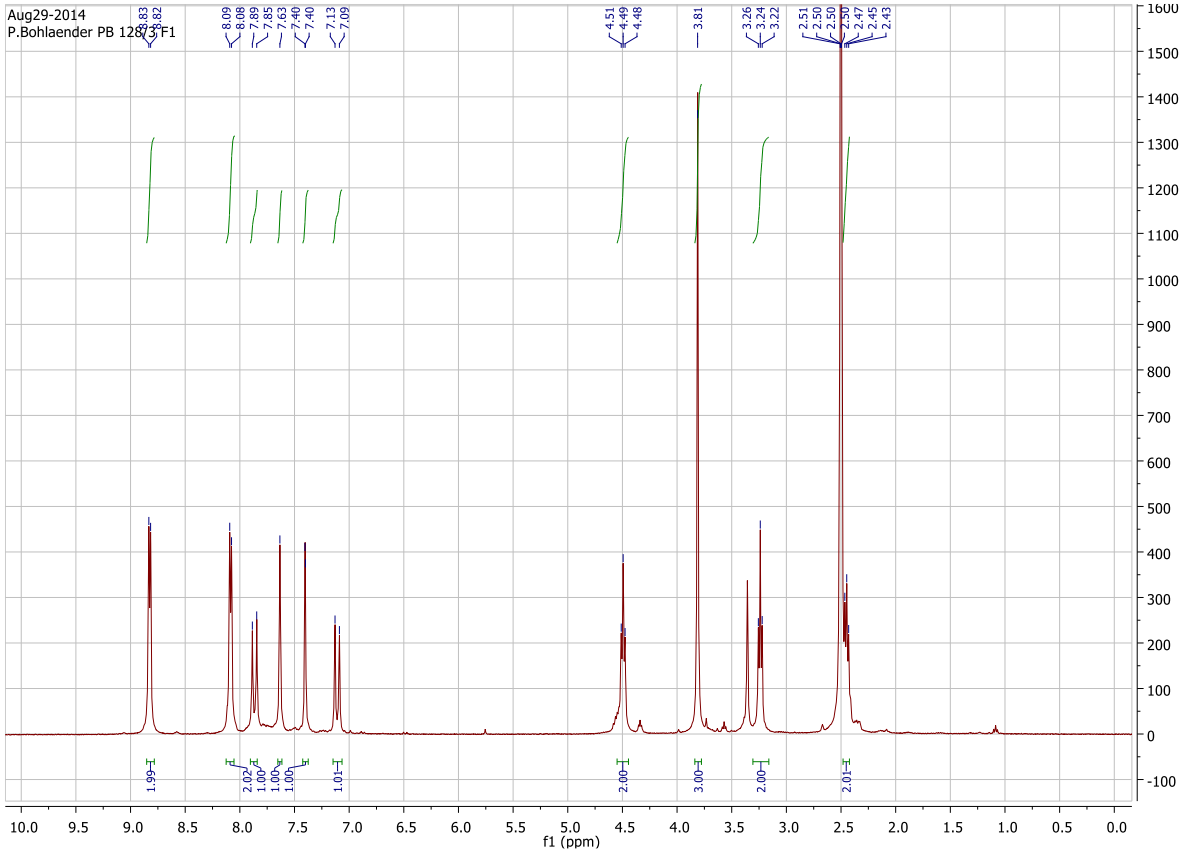
^{1}H-NMR (400MHz; DMSO-d_6):
δ (ppm) = 2.45 (t, *J* = 7.0, 2H), 3.24 (t, *J* = 7.0, 2H), 3.81 (s, 3H), 4.49 (t, *J* = 7.1, 2H), 7.11 (d, *J* = 16.1, 1H), 7.40 (s, 1H), 7.63 (s, 1H), 7.87 (d, *J* = 16.0, 1H), 8.08 (d, *J* = 6.5, 2H), 8.82 (d, *J* = 6.4, 2H).

^{13}C-NMR (100 MHz, DMSO-d_6):
δ (ppm) = 32.3, 34.0, 58.5, 104.2, 111.5, 116.4, 118.9, 119.7, 121.57, 129.5, 132.5, 142.6, 151.9.

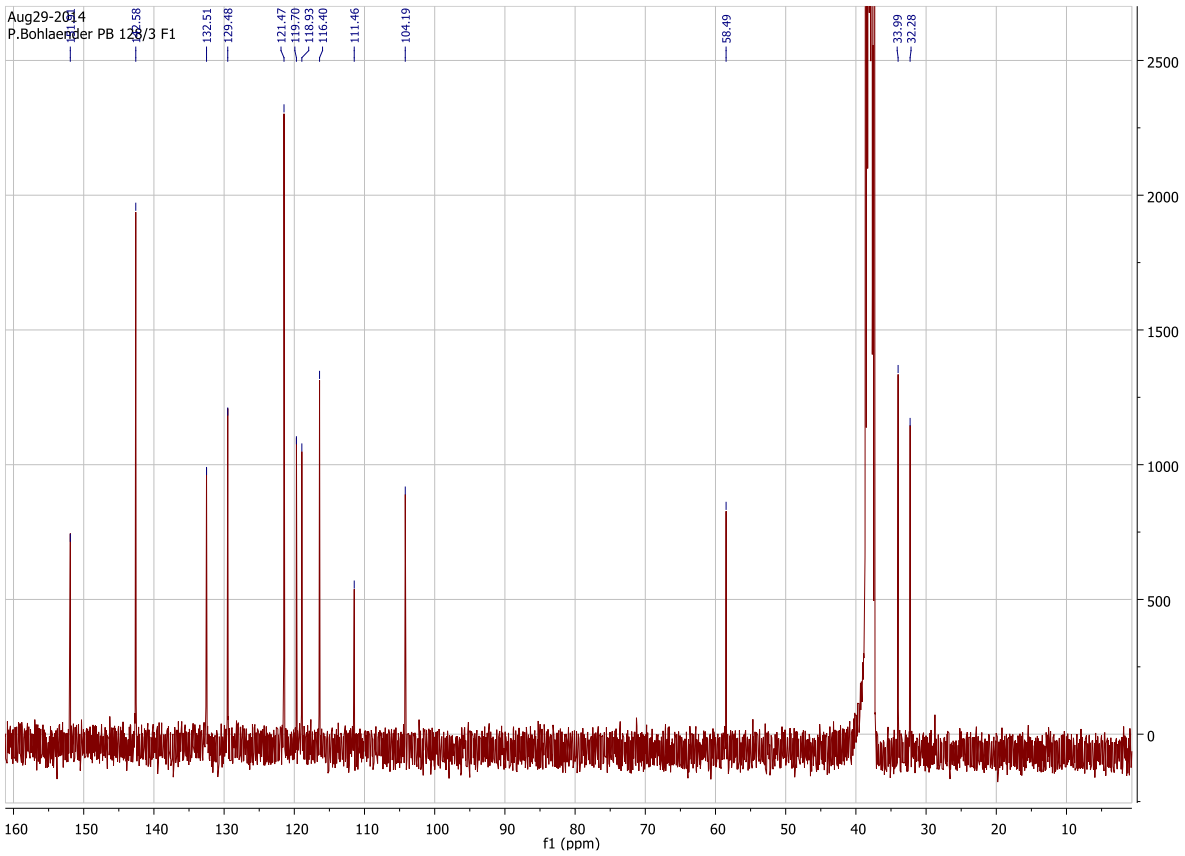
MS (FAB) m/z (%): 378.1 (60) [M^+].
HR-MS (FAB) m/z: calculated for $C_{16}H_{17}N_3I^+$ [M^+]: 378.0462, found: 378.0464.



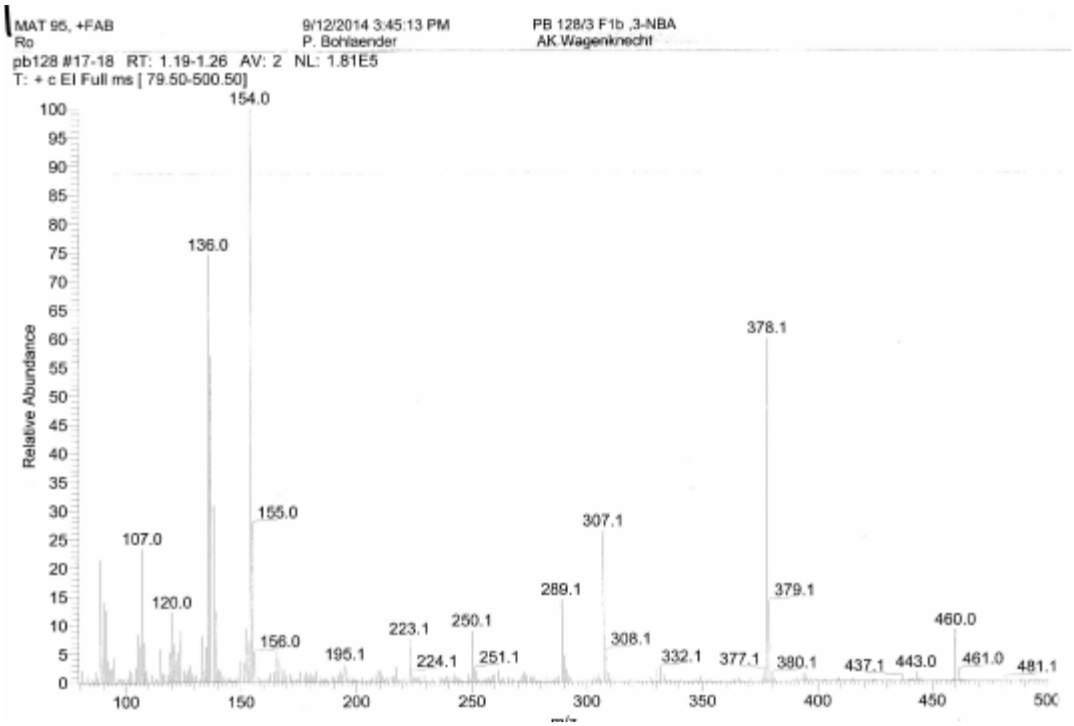
Scheme S15: IR of compound **8**.



Scheme S16: ^{1}H-NMR of compound **8**.



Scheme S17: ^{13}C-NMR of compound **8**.



Scheme S18: MS (FAB) of compound **8**.

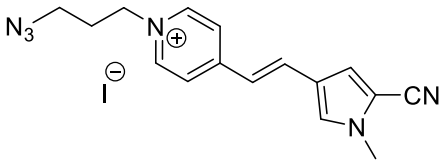
pb128-c5#33 RT: 2.31
T: + c EI Full ms [79.42-500.42]
m/z= 377.9507-378.0892

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
378.0464	778231.0	100.00	378.0462	0.18	$C_{16}H_{17}N_3{}^{127}I_1$

Scheme S19: HR-MS (FAB) of compound **8**.

4.4 Synthesis of azide 9:

(E)-1-(3-azidopropyl)-4-(2-(5-cyano-1-methyl-1H-pyrrol-3-yl)vinyl)pyridin-1-ium iodide



Under argon, a mixture of compound **8** (0.15 g, 0.30 mmol), NaN_3 (0.20 g, 3.00 mmol) and NaI (0.15 g, 1.00 mmol) in 3 mL dimethylformamide was stirred in a headspace vial at room temperature for 19 h. Afterwards the mixture was poured in 150 mL diethylether and 50 mL hexane. The precipitation was collected and washed three times with diethylether. After addition of 2 g NaI the crude product was solubilized in 50 mL water and 150 mL dichloromethane. The aqueous phase was extracted two times with 50 mL dichloromethane. The solvent of the organic phase was removed at 35°C and reduced pressure. The residue was solubilized in 3 mL methanol and 12 mL acetone (use of ultra sonic bath) and was diluted with 120 mL diethylether und 100 mL hexane. Most of diethylether was removed at 35°C and reduced pressure. The residue was suspended by use of ultra sonic bath. The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields a light-brown solid (82 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.12.

DC (2-Butanol : Wasser : Essigsäure = 80 : 15 : 5): R_f = 0.12.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3006 (w), 2219 (s), 2074 (s), 1611 (s), 1150 (m).

^{1}H-NMR (400MHz; DMSO-d_6):

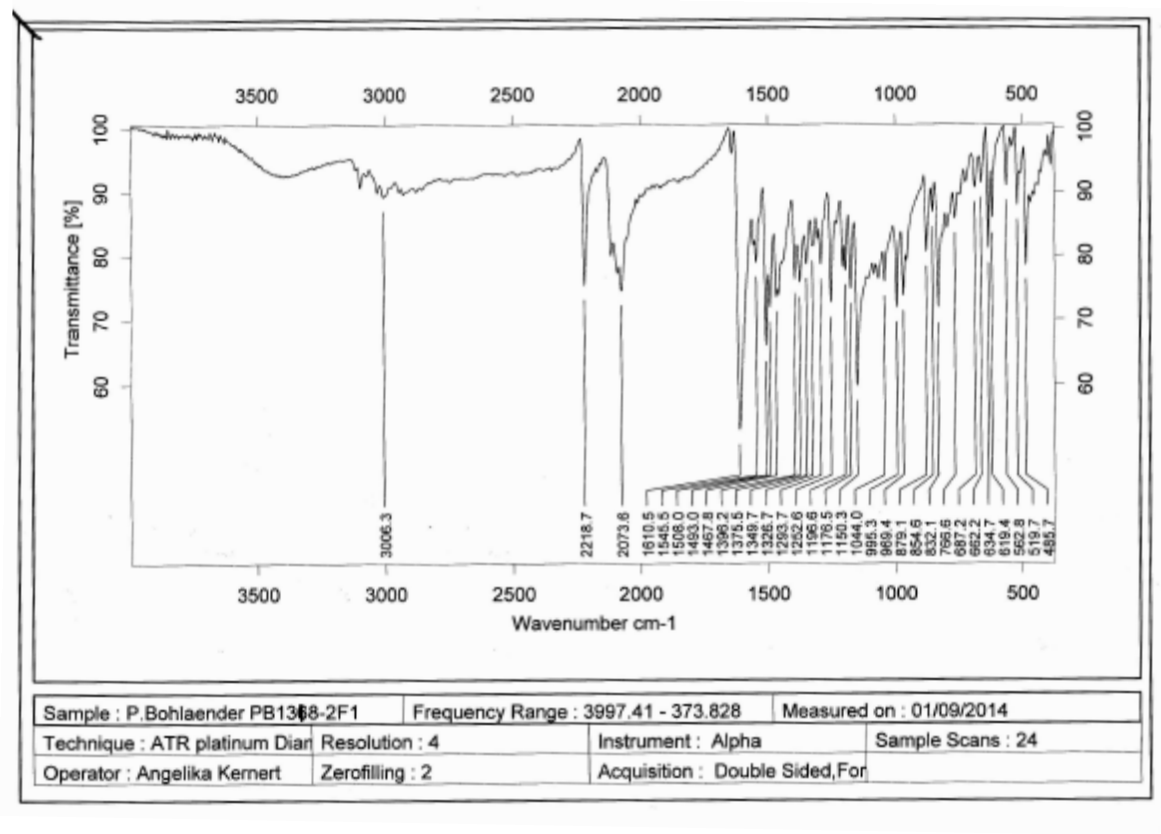
δ (ppm) = 2.17 (p, *J* = 6.9, 2H), 3.47 (t, *J* = 6.4, 2H), 3.81 (s, 3H), 4.52 (t, *J* = 7.1, 2H), 7.12 (d, *J* = 16.1, 1H), 7.41 (s, 1H), 7.64 (s, 1H), 7.88 (d, *J* = 16.1, 1H), 8.10 (d, *J* = 6.6, 2H), 8.86 (d, *J* = 6.6, 2H).

^{13}C-NMR (100 MHz, DMSO-d_6):

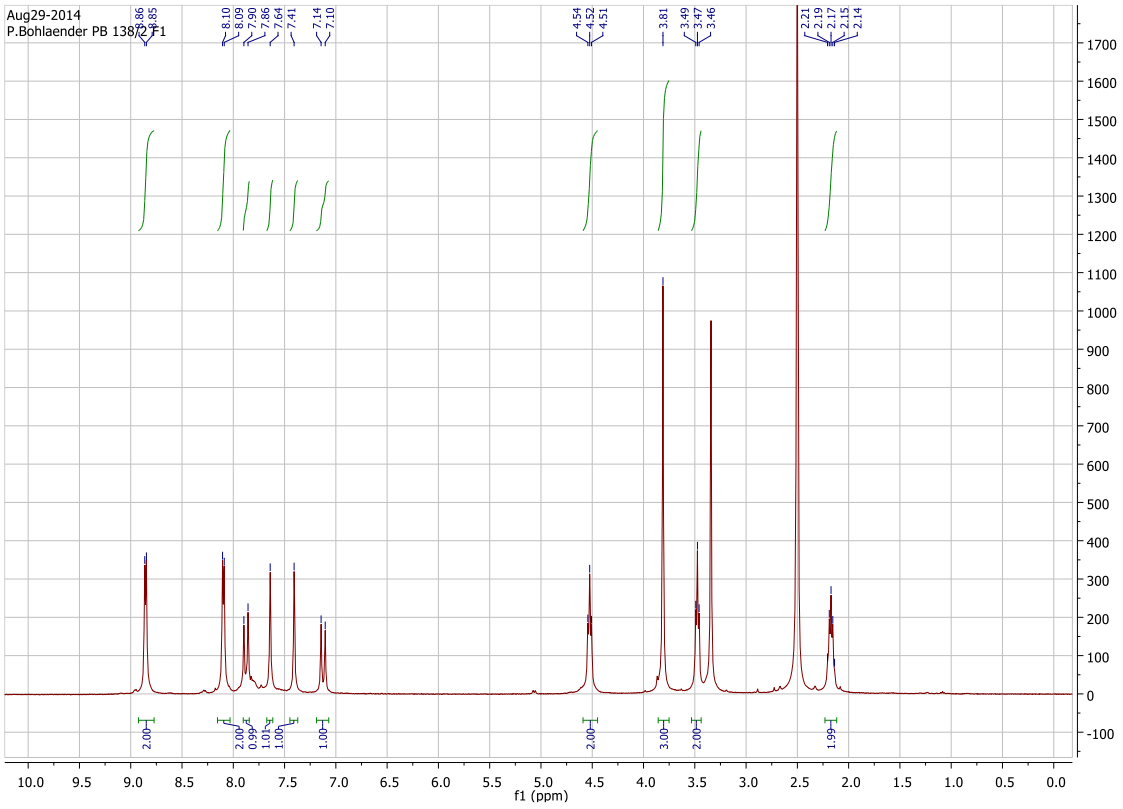
δ (ppm) = 29.5, 35.5, 47.6, 57.2, 105.7, 113.0, 117.9, 120.5, 121.2, 122.9, 131.0, 134.0, 144.1, 153.3.

MS (FAB) m/z (%): 293.2 (95) [M^+].

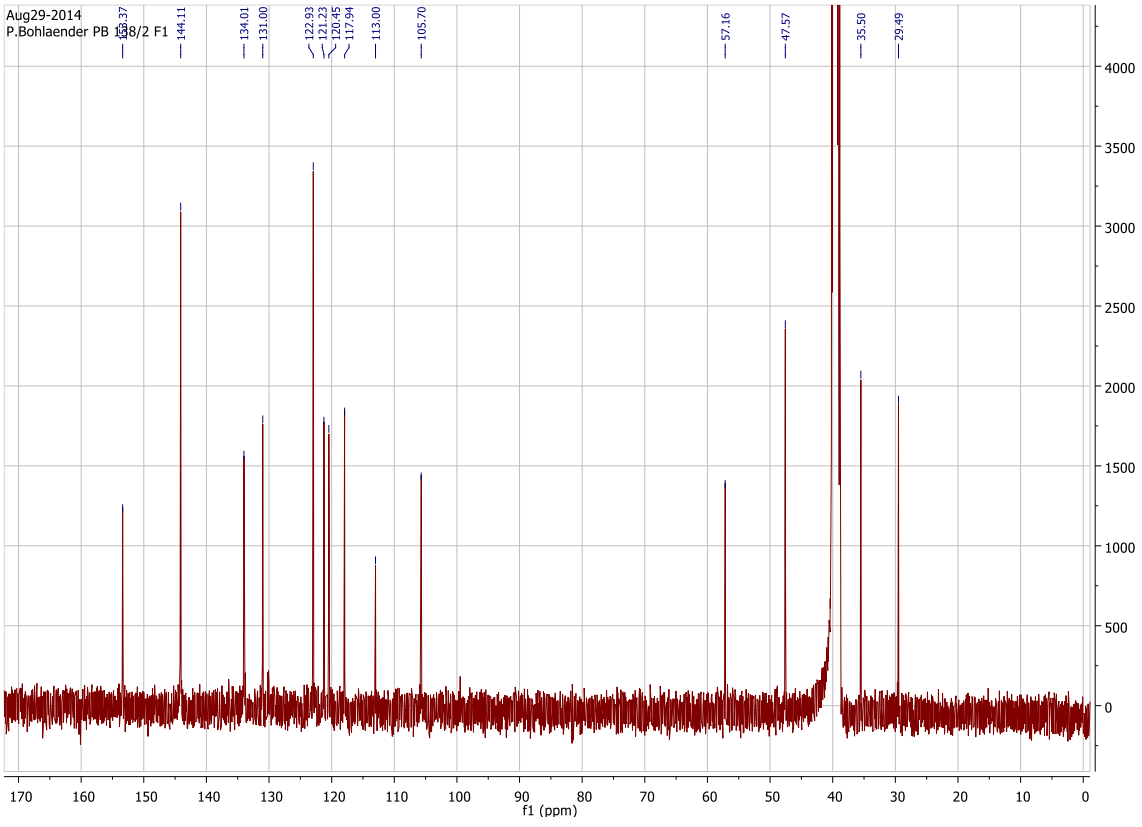
HR-MS (FAB) m/z: calculated for $C_{16}H_{17}N_6^+$ [M^+]: 293.1509, found: 293.1508.



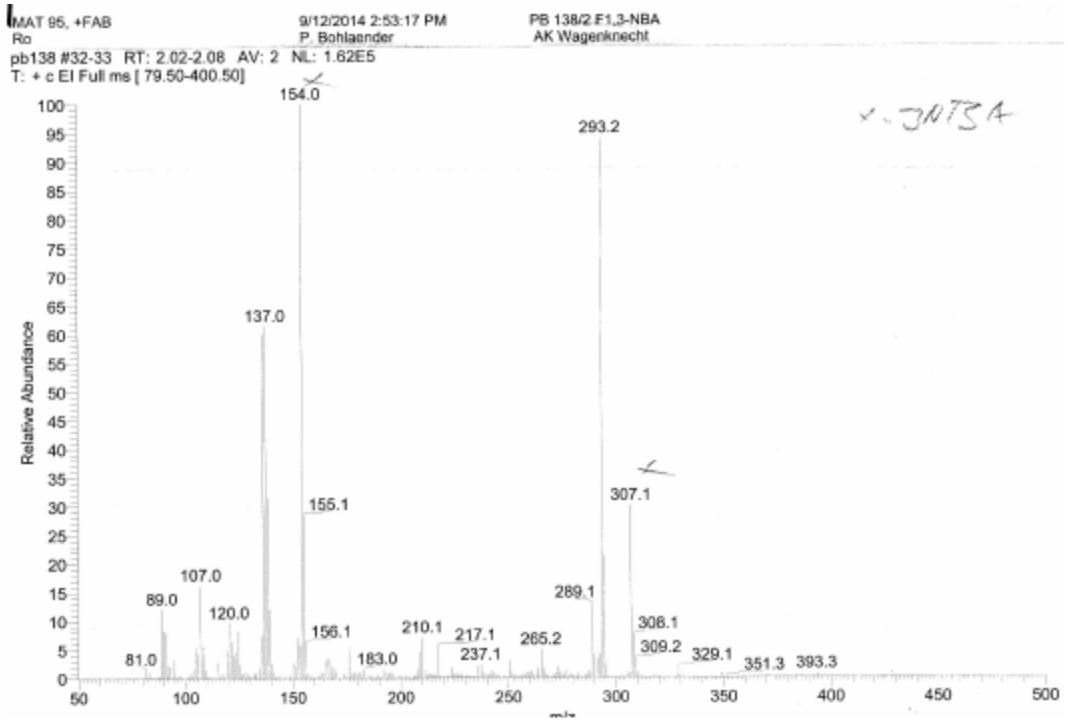
Scheme S20: IR of azide **9**.



Scheme S21: ^{1}H-NMR of azide **9**.



Scheme S22: ^{13}C-NMR of azide **9**.



Scheme S23: MS (FAB) of azide **9**.

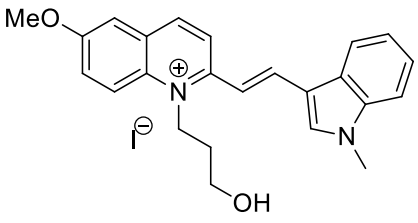
pb138-c2#9 RT: 0.60
T: + c EI Full ms [79.49-400.49]
m/z= 292.6724-293.6957

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
293.1508	538999.0	100.00	293.1509	-0.09	$C_{16}H_{17}N_6$

Scheme S24: HR-MS (FAB) of azide **9**.

4.5 Synthesis of dye 2:

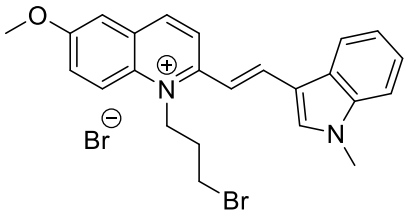
(E)-1-(3-hydroxypropyl)-6-methoxy-2-(2-(1-methyl-1H-indol-3-yl)vinyl)quinolin-1-ium iodide



Synthesis of dye 2 is already published.[1]

4.6 Synthesis of compound 10:

(E)-1-(3-brompropyl)-6-methoxy-2-(2-(1-methyl-1H-indole-3-yl)vinyl)quinolin-1-ium bromide



Under argon, a mixture of dye **2** (0.25 g, 0.50 mmol), triphenylphosphine (0.39 g, 1.50 mmol) and tetrabromomethane (0.55 g, 1.65 mmol) in 5 mL dichloromethane was stirred in a headspace vial at room temperature for 2 h. After addition of 0.1 g NaBr to the mixture it was solubilized in 75 mL acetone and 10 mL methanol and the solvent was removed at 50°C and reduced pressure reduced to a residual volume of 15 mL. The suspension was diluted with 5 mL methanol and the product was crystallized by use of ultra sonic bath. The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields an orange solid (75 %).

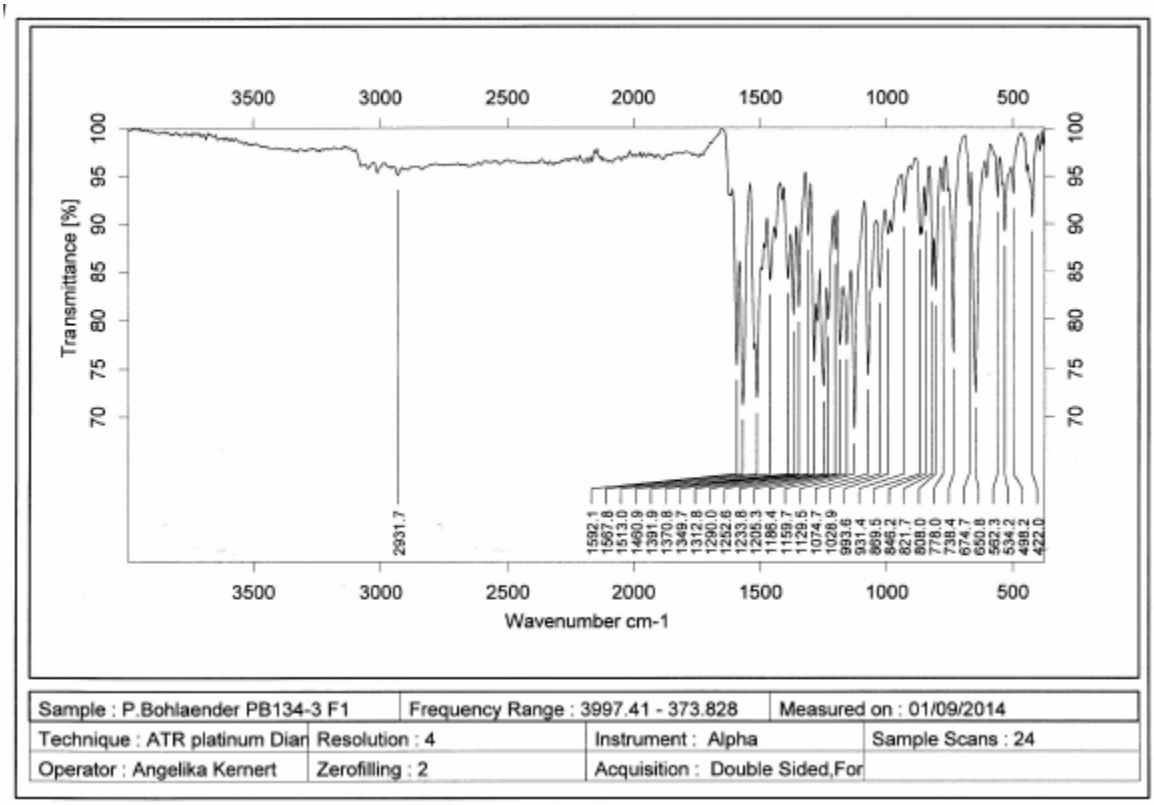
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.36.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 1592 (m), 1568 (m), 1513 (m), 1350 (w).

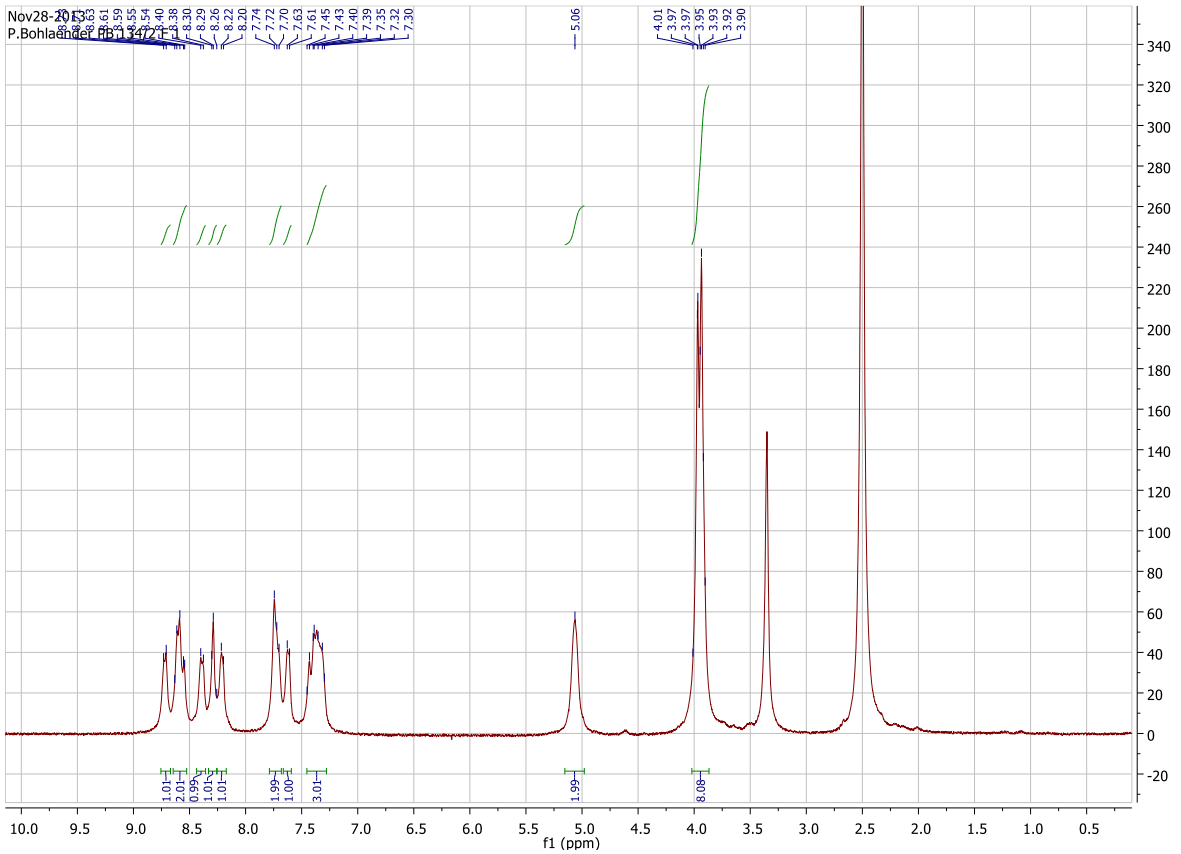
^{1}H-NMR (400MHz; DMSO-d_6):
δ (ppm) = 3.87 – 4.02 (m, 8H), 4.98 - 5.15 (m, 2H), 7.28 – 7.45 (m, 3H), 7.62 (d, *J* = 8.1, 1H), 7.68 - 7.79 (m 2H), 8.21 (d, *J* = 7.6, 1H), 8.26 – 8.33 (m, 1H), 8.39 (d, *J* = 9.6, 1H), 8.57 (dd, *J* = 20.6, 7.5, 2H), 8.72 (d, *J* = 9.4, 1H).

^{13}C-NMR (100 MHz, DMSO-d_6):
δ (ppm) = 30.8, 31.3, 33.5, 48.8, 56.1, 109.2, 110.4, 111.3, 113.3, 120.0, 120.4, 120.5, 122.0, 123.4, 125.0, 125.5, 128.8, 133.5, 137.2, 138.1, 141.1, 141.5, 153.8, 158.1.

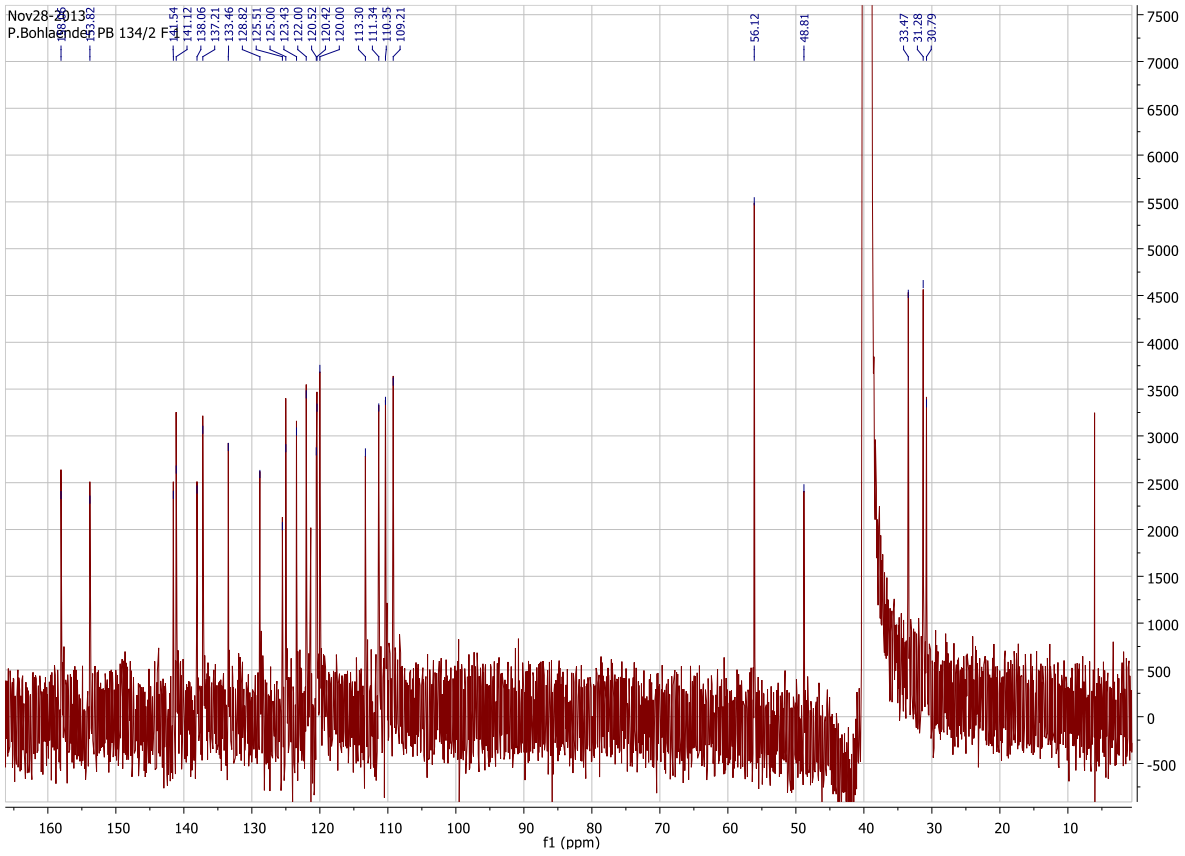
MS (FAB) m/z (%): 435.0 (50) [M^+].
HR-MS (FAB) m/z: calculated for $C_{24}H_{24}N_2Br^+$ [M^+]: 435.1067, found: 435.1066.



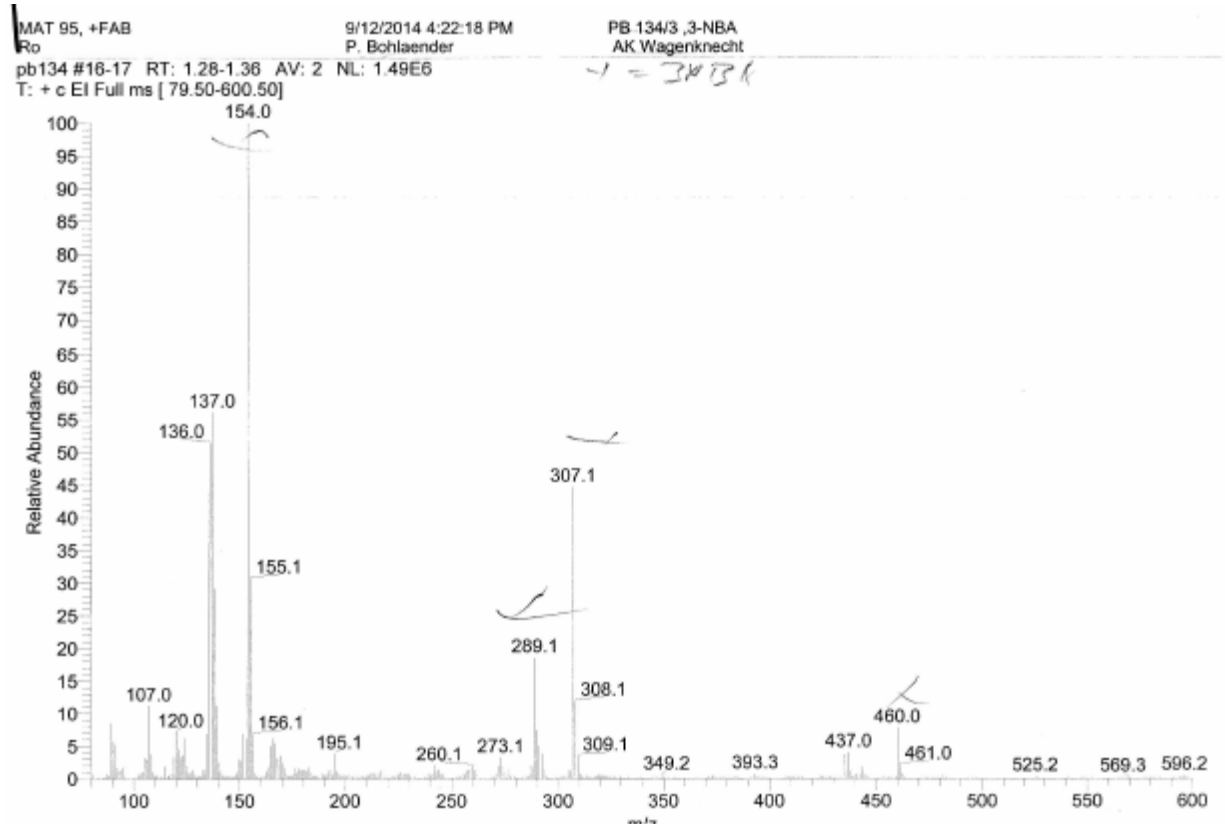
Scheme S25: IR of compound **10**.



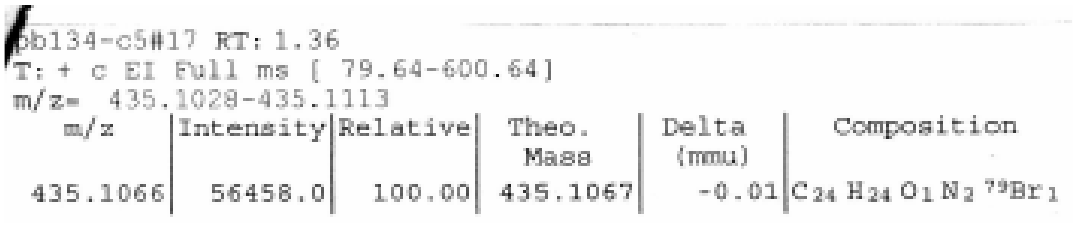
Scheme S26: ^{1}H-NMR compound **10**.



Scheme S27: ^{13}C-NMR of compound **10**.



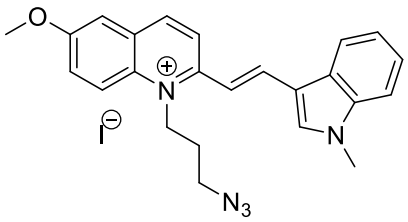
Scheme S28: MS (FAB) of compound **10**.



Scheme S29: HR-MS (FAB) of compound **10**.

4.7 Synthesis of azide 11:

(E)-1-(3-azidopropyl)-6-methoxy-2-(2-(1-methyl-1H-indole-3-yl)vinyl)quinolin-1-ium iodide



Under argon, a mixture of compound **10** (0.16 g, 0.30 mmol), NaN_3 (0.20 g, 3.00 mmol) and NaI (0.15 g, 1.00 mmol) in 6 mL dimethylformamide was stirred in a headspace vial at room temperature for 19 h. Afterwards the mixture was poured in 150 mL diethylether and 50 mL hexane. The precipitation was collected and washed three times with diethylether. After addition of 2 g NaI the crude product was solubilized in 50 mL water and 150 mL dichloromethane. The aqueous phase was extracted four times with 50 mL dichloromethane. The solvent of the organic phase was removed at 35°C and reduced pressure. The residue was suspended in 10 mL methanol (use of ultra sonic bath). The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields an orange solid (72 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.32.

DC (2-Butanol : Wasser : Essigsäure = 80 : 15 : 5): R_f = 0.32.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3056 (w), 2097 (s), 1592 (m), 1567 (m), 1511 (w), 1350 (w).

^{1}H-NMR (400MHz; DMSO-d_6):

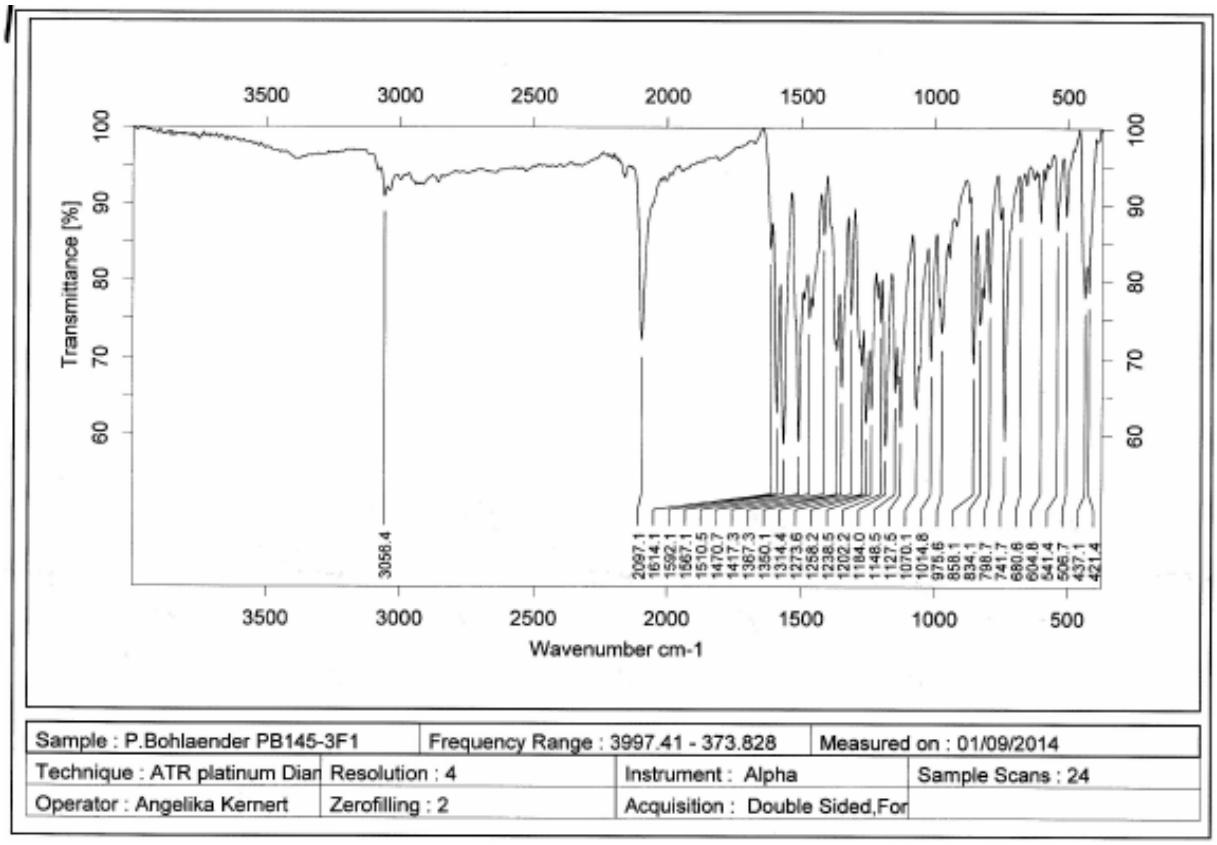
δ (ppm) = 2.12 – 2.24 (m, 2H), 3.78 (t, *J* = 6.1, 2H), 3.94 (s, 3H), 3.97 (s, 3H), 4.92 – 5.07 (m, 2H), 7.31 – 7.46 (m, 3H), 7.64 (d, *J* = 8.0, 1H), 7.68 – 7.77 (m, 2H), 8.20 – 8.30 (m, 2H), 8.40 (d, *J* = 9.8, 1H), 8.51 – 8.62 (m, 2H), 8.72 (d, *J* = 9.2, 1H).

^{13}C-NMR (100 MHz, DMSO-d_6):

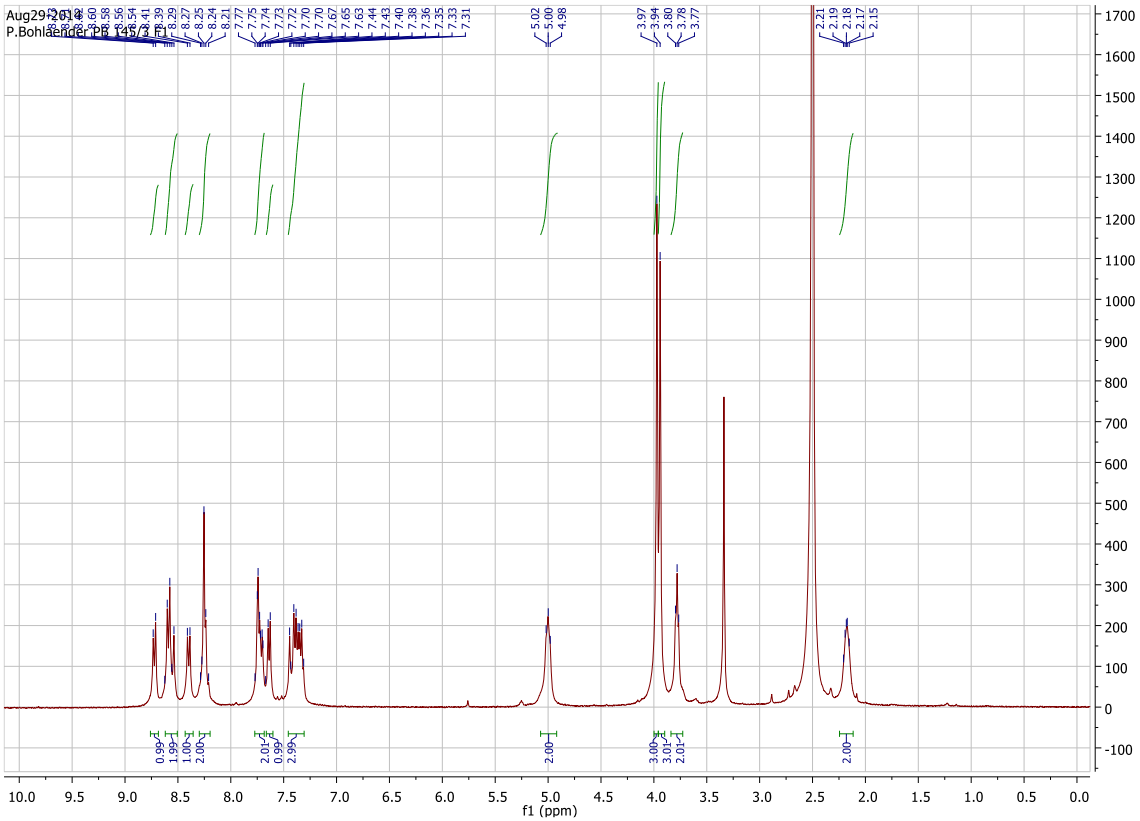
δ (ppm) = 27.5, 33.4, 47.5, 48.0, 56.1, 109.2, 110.4, 111.36, 113.3, 120.1, 120.3, 120.4, 122.1, 123.4, 125.0, 125.4, 128.8, 133.4, 137.6, 138.2, 141.1, 141.5, 153.7, 158.1.

MS (FAB) m/z (%): 398.3 (300) [M^+].

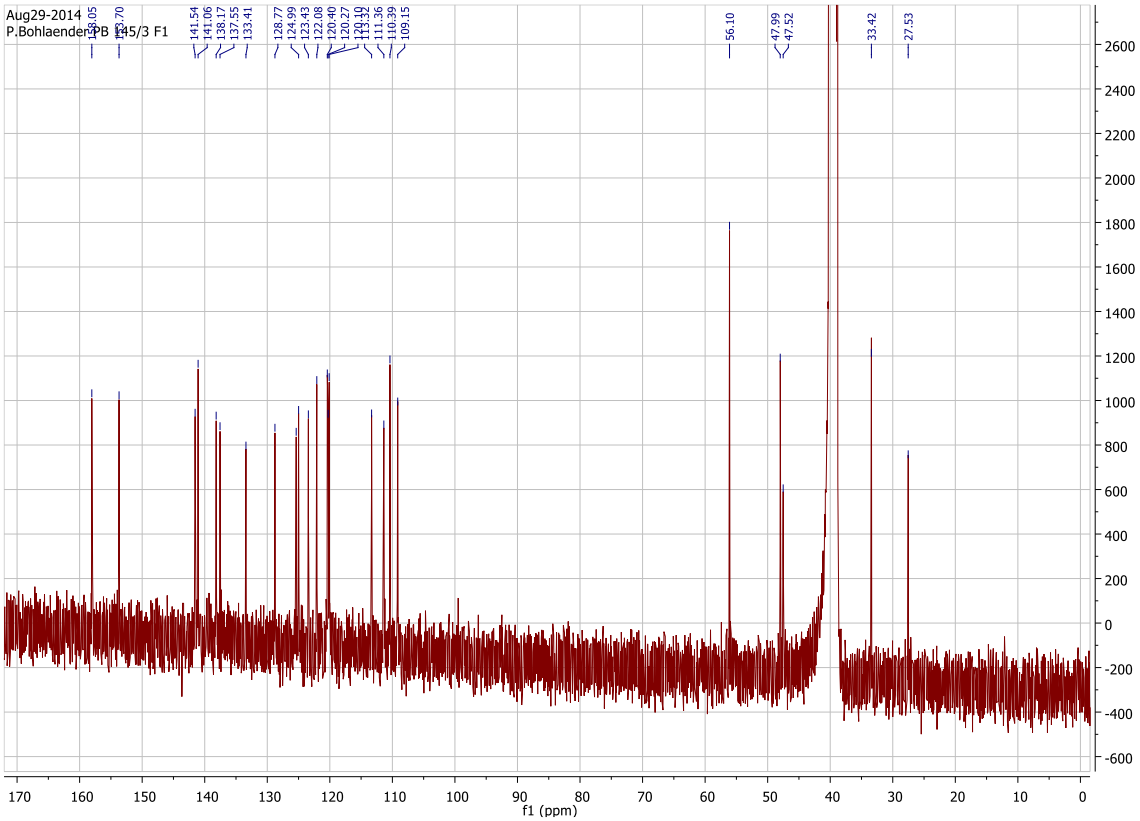
HR-MS (FAB) m/z: calculated for $C_{24}H_{24}ON_5^+$ [M^+]: 398.1975, found: 398.1974.



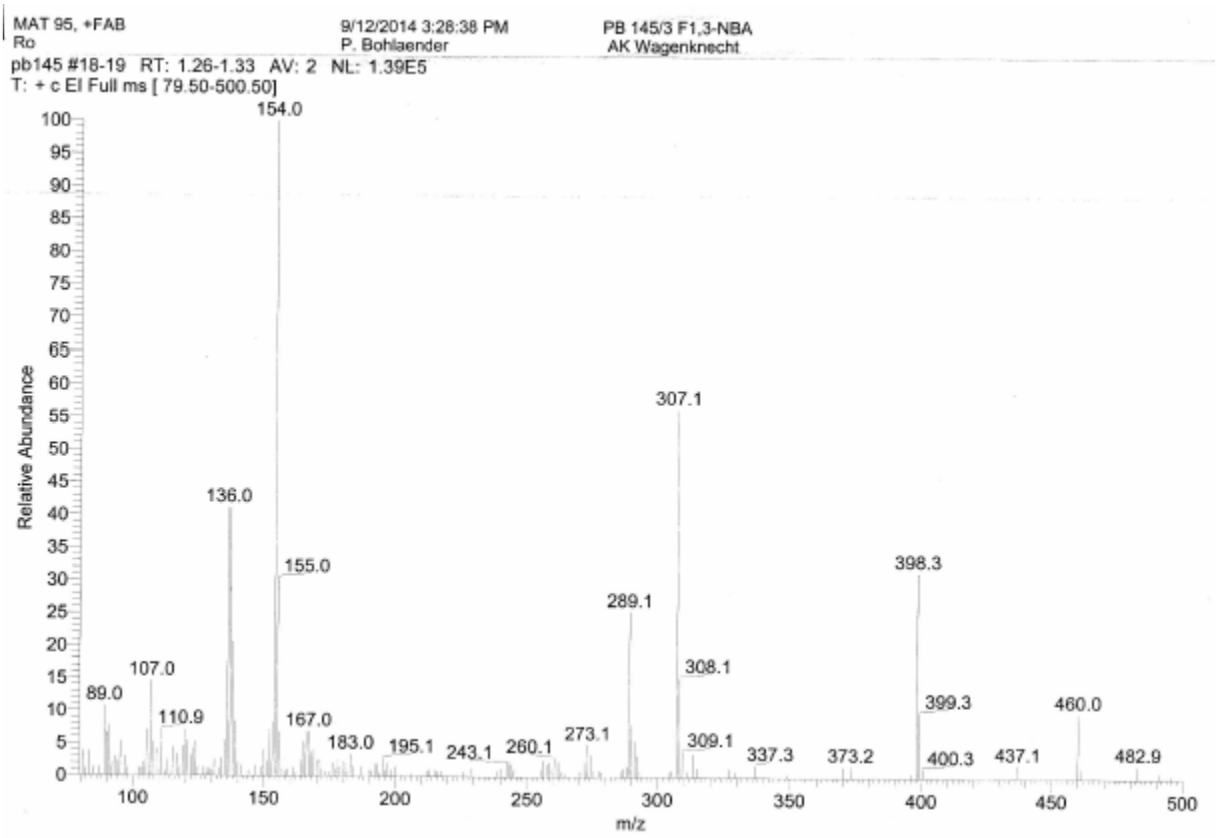
Scheme S30: IR of azide **11**.



Scheme S31: ^{1}H-NMR of azide **11**.



Scheme S32: ^{13}C-NMR of azide **11**.



Scheme S33: MS (FAB) of azide **11**.

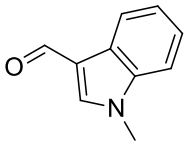
pb145-c8#7 RT: 0.49
T: + c EI Full ms [79.44-500.44]
m/z= 398.1580-398.2516

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
398.1974	1313.0	100.00	398.1975	-0.16	$C_{24}H_{24}O_1N_5$

Scheme S34: HR-MS (FAB) of azide **11**.

4.8 Synthesis of compound 12:

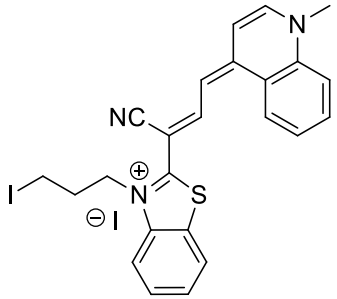
1-methyl-1H-indole-3-carbaldehyde



Synthesis of compound 12 is already published.[1]

4.9 Synthesis of dye 3:

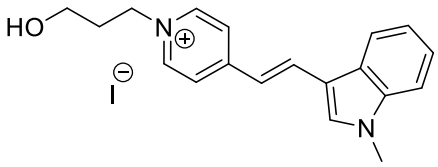
bis(2-((1E,3Z)-1-cyano-3-(1-methylquinolin-4(1H)-ylidene)prop-1-en-1-yl)-3-(3-iodopropyl)benzo[d]thiazol-3-ium) monoiodide hydroiodide



Synthesis of dye 3 and the corresponding azide is already published.[2]

4.10 Synthesis of dye 4:

(E)-1-(3-hydroxypropyl)-4-(2-(1-methyl-1H-indole-3-yl)vinyl)pyridin-1-ium iodide



Under argon, to a mixture of compound **6** (0.28 g, 1.00 mmol) and compound **12** (0.34 g, 2.11 mmol) in 13 mL ethanol, piperidine (0.22 mL, 0.19 g, 2.20 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 20 h. After cooling to room temperature the precipitated product was collected and washed three times with diethyl ether. Drying under reduced pressure yields an orange solid (69 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.18.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3333 (m), 1594 (s), 1504 (m), 1307 (w), 1173 (m).

^{1}H-NMR (400MHz; DMSO-d_6):

δ (ppm) = 2.05 (p, J = 6.6, 2H), 3.46 (q, J = 5.5, 2H), 3.88 (s, 3H), 4.49 (t, J = 7.0, 2H), 4.76 (t, J = 4.8, 1H), 7.25 – 7.36 (m, 3H), 7.57 (d, J = 8.0, 1H), 7.97 (s, 1H), 8.12 – 8.27 (m, 4H), 8.75 (d, J = 6.5, 2H).

^{13}C-NMR 100 MHz, DMSO-d_6):

δ (ppm) = 33.1, 56.8, 57.1, 111.0, 112.6, 116.8, 120.6, 121.4, 121.8, 123.0, 125.3, 135.7, 135.8, 138.0, 143.5, 154.3.

MS (FAB) m/z (%): 293.2 (100) [M^+].

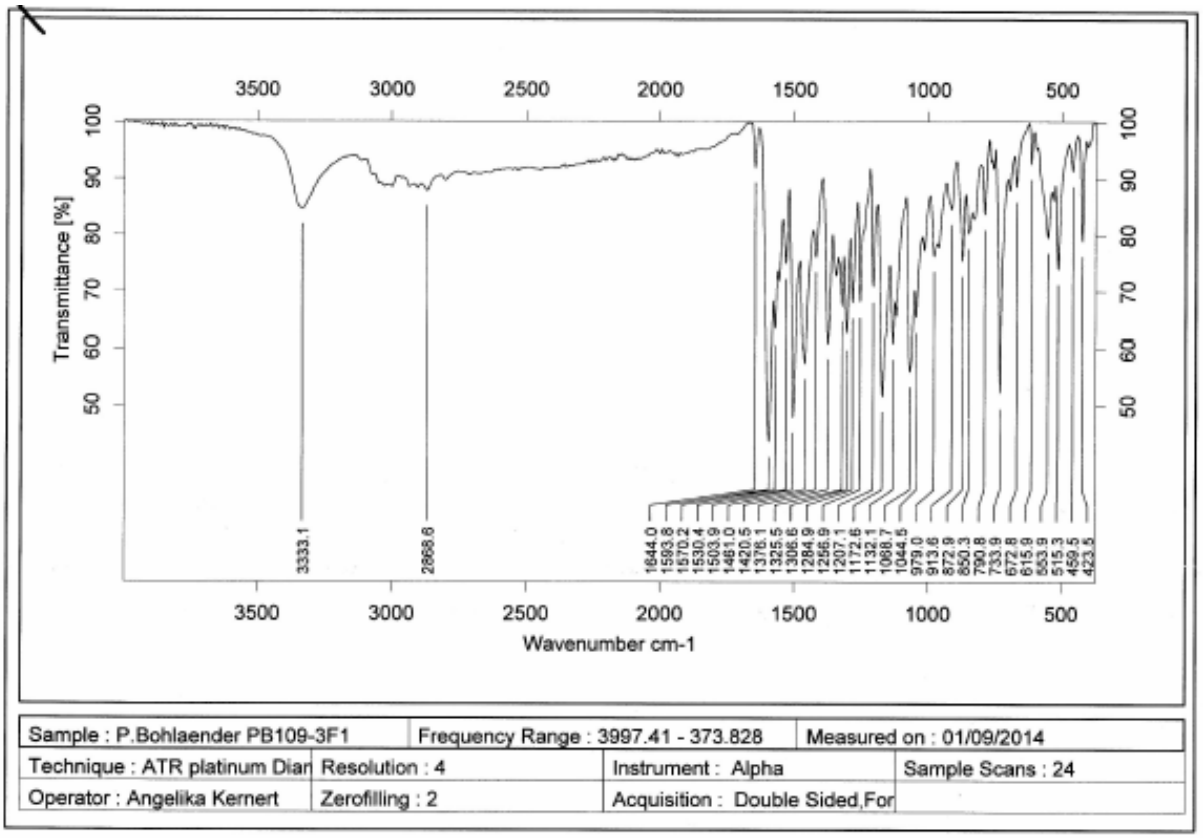
HR-MS (FAB) m/z: calculated for $C_{19}H_{21}N_2O$ [M^+]: 293.1648, found: 293.1648.

Elementary analysis

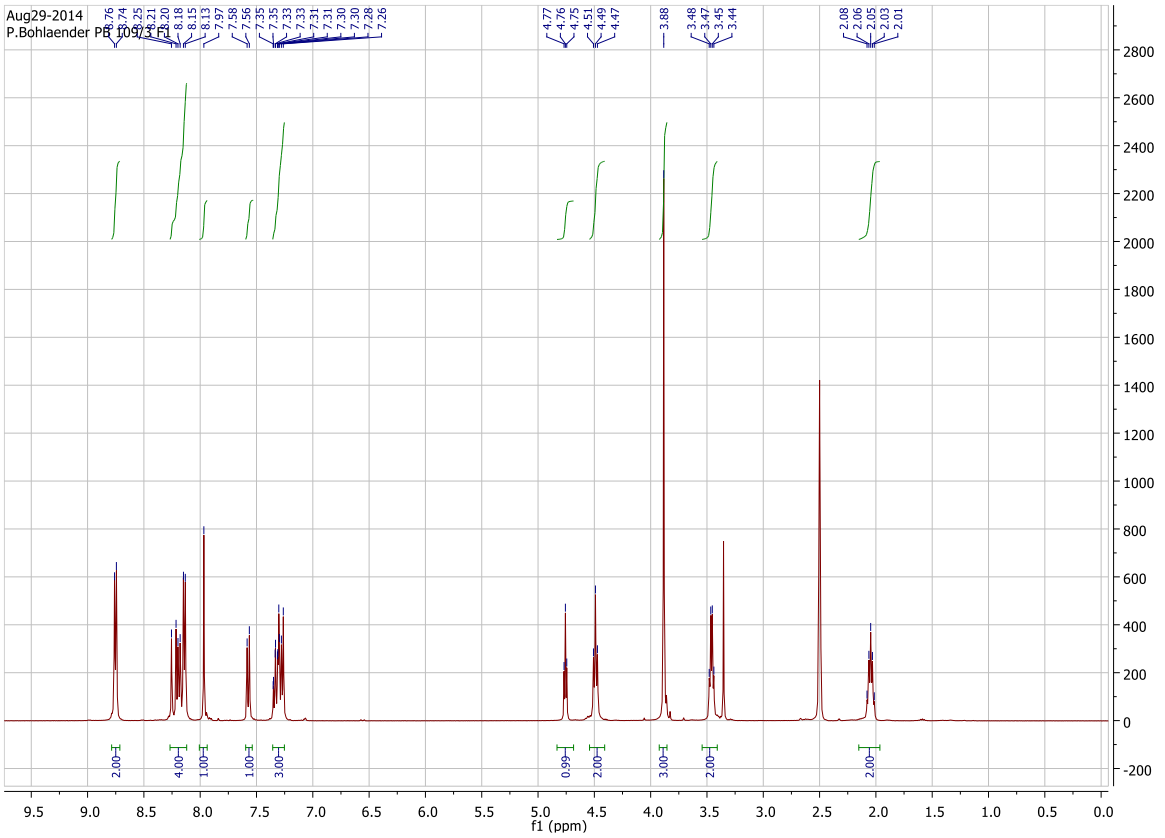
calculated for $C_{19}H_{21}IN_2O$:

N: 6.67 % → found: 6.58 % C: 54.30 % → found: 53.99 %

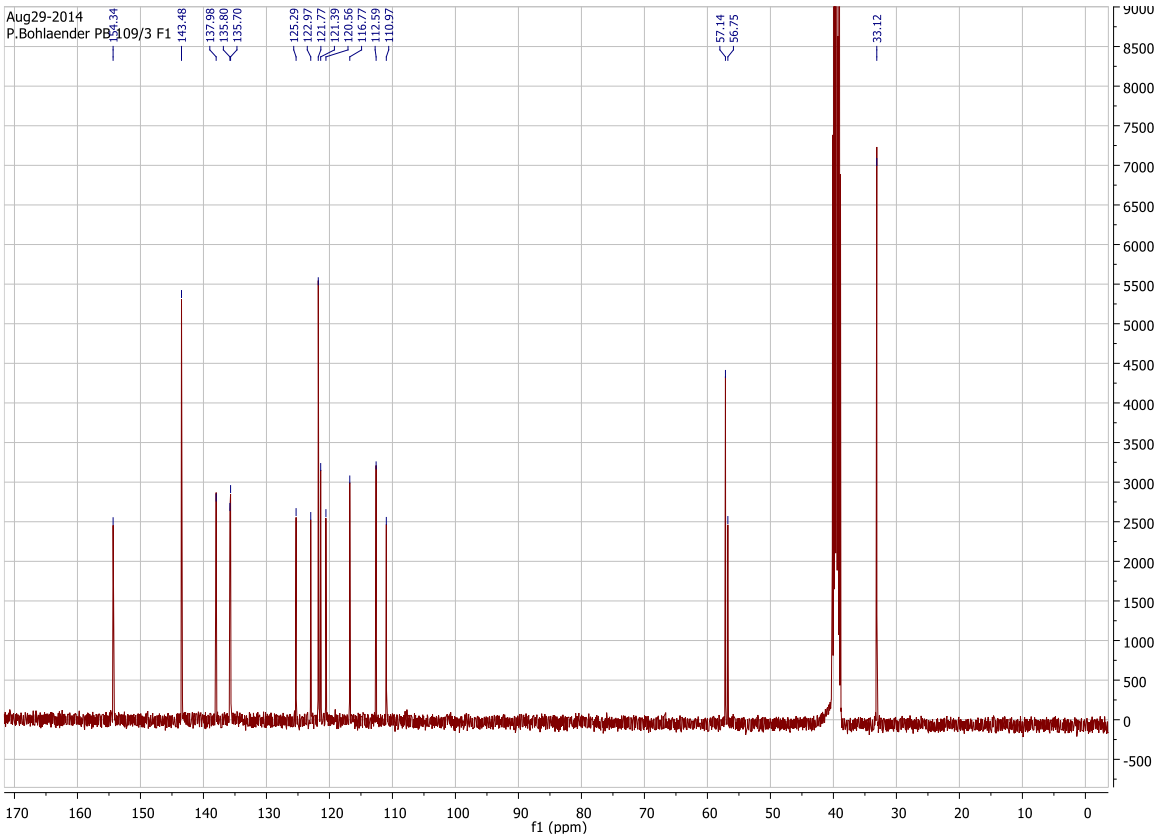
H: 5.04 % → found: 5.05 %



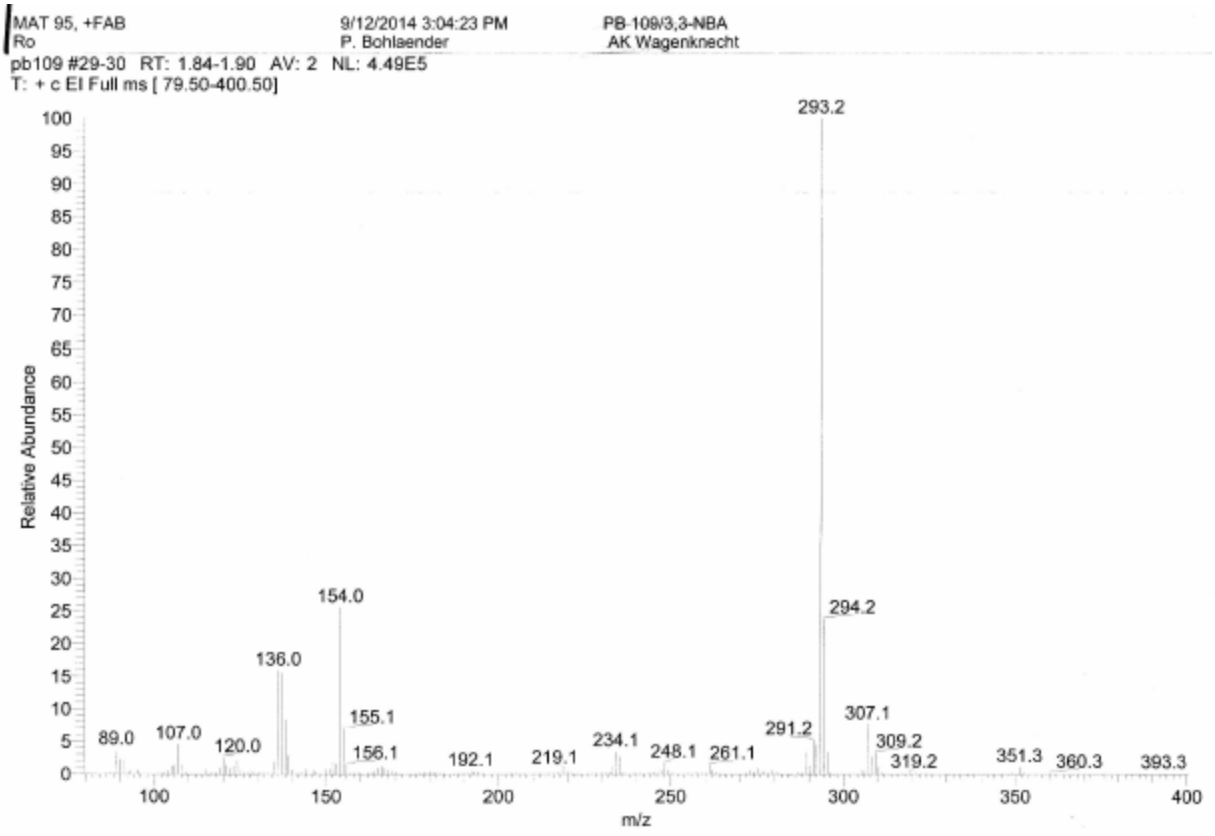
Scheme S35: IR of dye **4**.



Scheme S36: ^{1}H-NMR dye **4**.



Scheme S37: ^{13}C-NMR of dye **4**.



Scheme S38: MS (FAB) of dye **4**.

pb109-c1#13 RT: 0.85
T: + c EI Full ms [79.49-400.49]
m/z= 292.5207-293.5558

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
293.1648	373954.0	100.00	293.1648	-0.06	$C_{19}H_{21}O_1N_2$

Scheme S39: HR-MS (FAB) of dye **4**.

Berechnet: N: [illegible] C: [illegible] H: [illegible] S: [illegible]

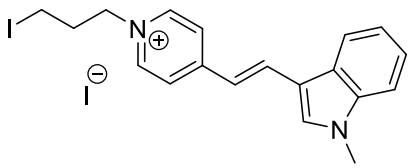
Gefunden: N: 6,58 C: 53,93 H: 5,04 S:

Gefunden: N: 6,58 C: 53,99 H: 5,05 S:

Scheme S40: Elementary analysis of dye **4**.

4.11 Synthesis of compound 13:

(E)-1-(3-iodopropyl)-4-(2-(1-methyl-1H-indole-3-yl)vinyl)pyridin-1-ium iodide



Under argon, a mixture of dye **4** (0.21 g, 0.50 mmol), triphenylphosphine (0.39 g, 1.50 mmol) and tetrabromomethane (0.55 g, 1.65 mmol) in 5 mL dichloromethane was stirred in a headspace vial at room temperature for 2 h. After addition of 2.5 g NaI to the mixture it was solubilized with 75 mL acetone and 10 mL methanol and the solvent was removed at 50°C and reduced pressure. The residue was dissolved in 20 mL acetone and 8 mL methanol and stirred at 50°C for 90 h. After cooling to room the precipitation was removed and the filtrate was reduced at 40°C and reduced pressure. The precipitation of the crude product was collected and washed three times with diethylether and solubilized in 150 mL dichloromethane and 50 mL water. The aqueous phase was extracted additional two times with 50 mL dichloromethane, respectively. The solvent was removed at 40°C and reduced pressure. The residue was suspended in 10 mL methanol using ultra sonic bath. The suspension was diluted with 10 mL methanol and the product was crystallized with use of ultra sonic bath. The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields an orange solid (95 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.33.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 1586 (m), 1567 (w), 1370 (w), 1169 (m).

^{1}H-NMR (400MHz; DMSO-d_6):

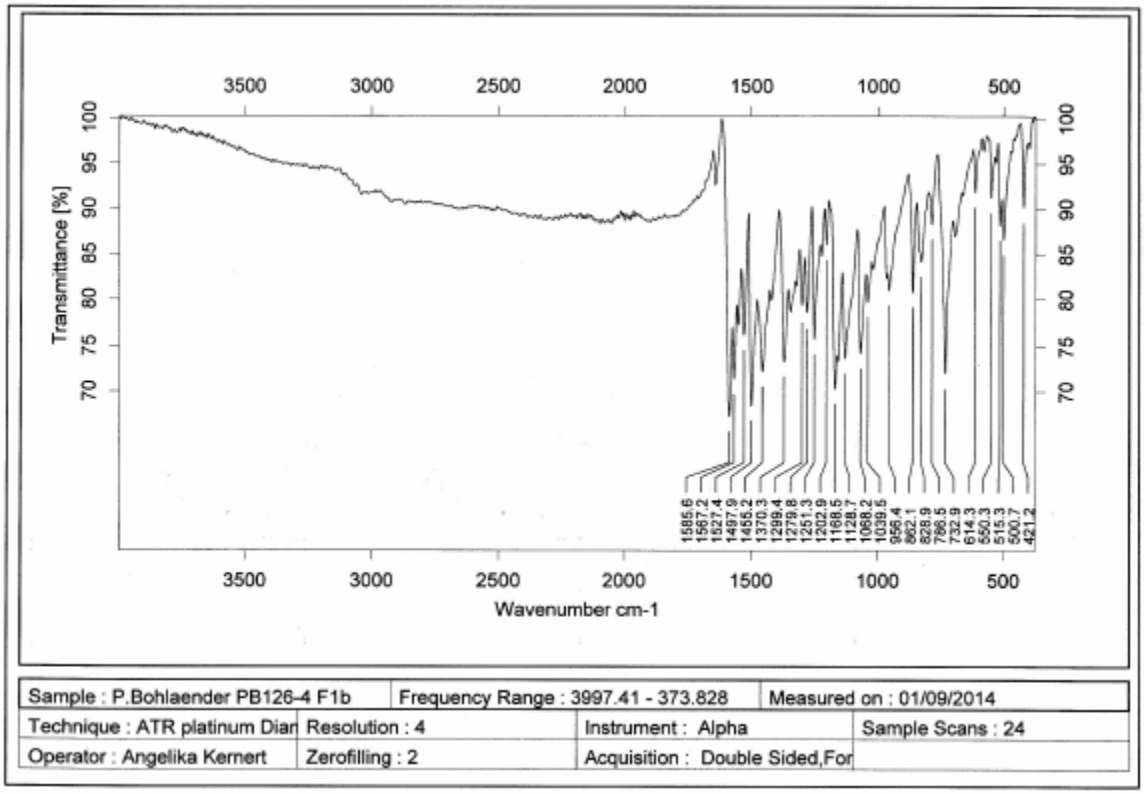
δ (ppm) = 2.42 – 2.48 (m, 2H), 3.25 (t, *J* = 7.0, 2H), 3.89 (s, 3H), 4.45 (t, *J* = 7.1, 2H), 7.25 – 7.36 (m, 3H), 7.58 (d, *J* = 8.0, 1H), 7.96 (s, 1H), 8.13 – 8.28 (m, 4H), 8.74 (d, *J* = 6.7, 2H).

^{13}C-NMR (100 MHz, DMSO-d_6):

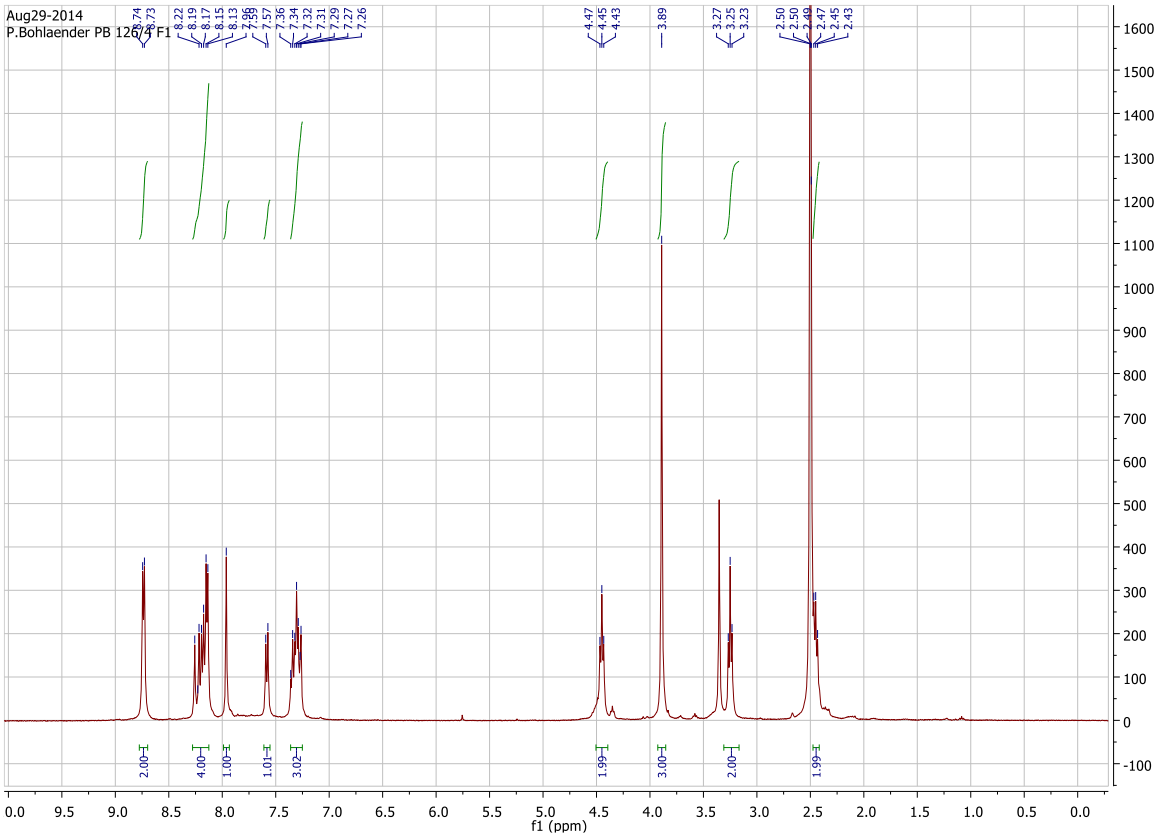
δ (ppm) = 31.5, 32.2, 57.9, 109.4, 111.0, 115.1, 118.9, 119.8, 120.3, 121.4, 123.7, 134.2, 134.4, 136.4, 141.8, 153.0.

MS (FAB) m/z (%): 403.2 (100) [M^+].

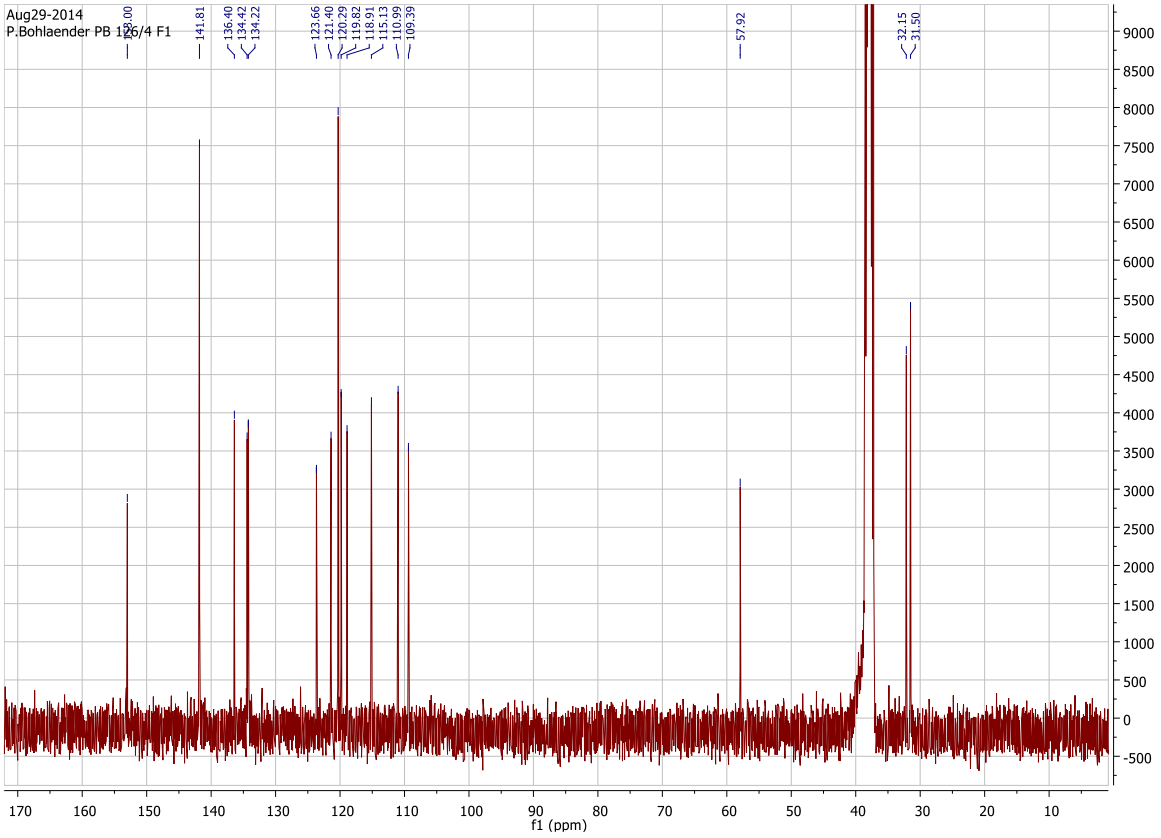
HR-MS (FAB) m/z: calculated for $C_{19}H_{20}N_2I^+$ [M^+]: 403.0666, found: 403.0667.



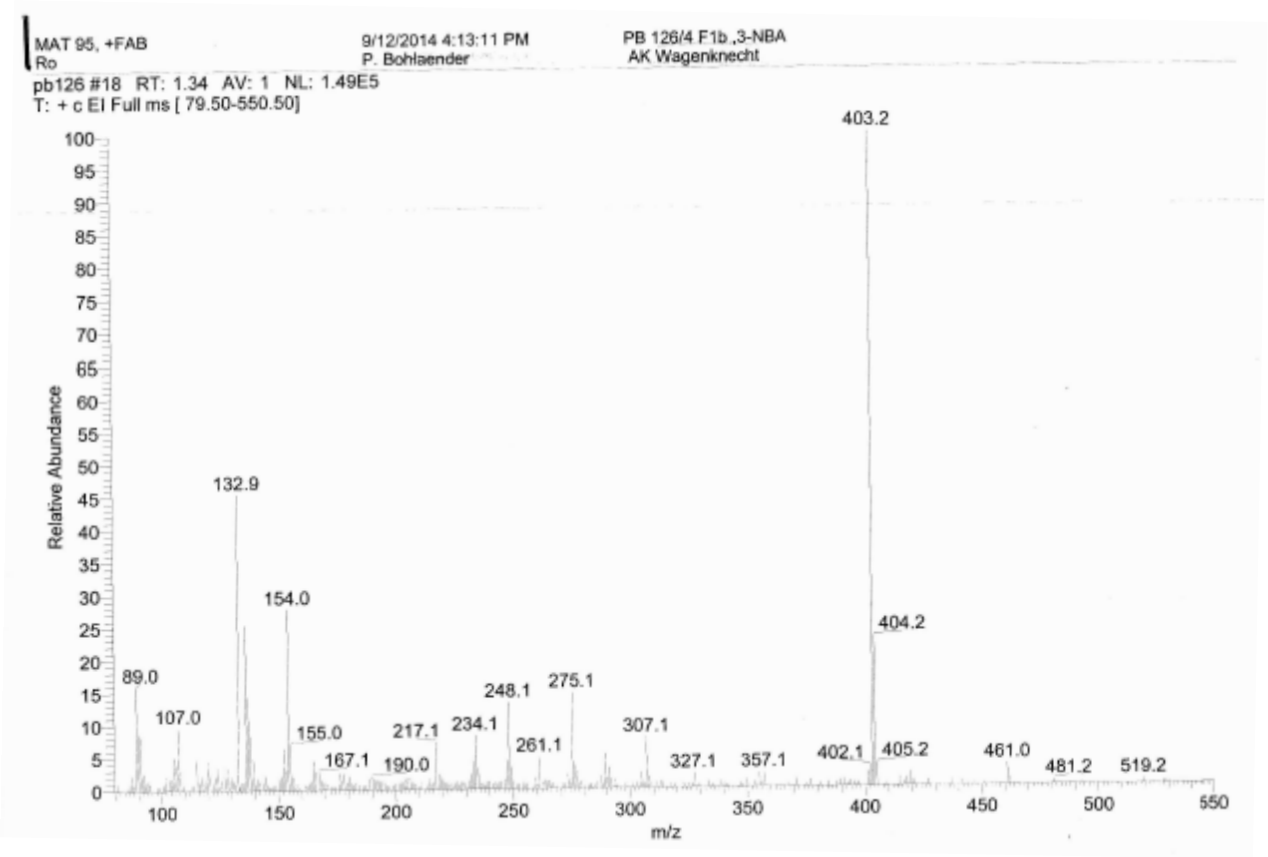
Scheme S41: IR of compound **13**.



Scheme S42: ^{1}H-NMR of compound **13**.



Scheme S43: ^{13}C-NMR of compound **13**.



Scheme S44: MS (FAB) of compound **13**.

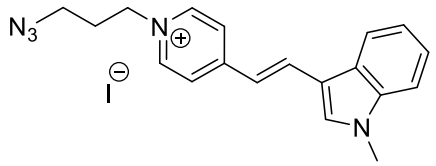
pb126-c2#6 RT: 0.46
T: + c EI Full ms [79.43-550.43]
m/z= 403.0663-403.0684

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
403.0667	747746.0	100.00	403.0666	0.17	$C_{19}H_{20}N_2{}^{127}I_1$

Scheme S45: HR-MS (FAB) of compound **13**.

4.12 Synthesis of azide 14:

(E)-1-(3-azidopropyl)-4-(2-(1-methyl-1H-indole-3-yl)vinyl)pyridin-1-ium iodide



Under argon, a mixture of compound **13** (0.16 g, 0.30 mmol), NaN_3 (0.20 g, 3.00 mmol) and NaI (0.15 g, 1.00 mmol) in 3 mL dimethylformamide was stirred in a headspace vial at room temperature for 19 h. Afterwards the mixture was poured in 100 mL diethylether and 100 mL hexane. The precipitation was collected and washed three times with diethylether. After addition of 2 g NaI the crude product was solubilized in 100 mL water and 100 mL dichloromethane. The aqueous phase was extracted two times with 50 mL dichloromethane. The solvent of the organic phase was removed at 35°C and reduced pressure. The residue was solubilized in 3 mL methanol and 12 mL acetone (use of ultra sonic bath) and was diluted with 120 mL diethylether und 100 mL hexane. Most of diethylether was removed at 35°C and reduced pressure. The residue was suspended by use of ultra sonic bath. The precipitation was collected and washed three times with diethylether. Drying under reduced pressure yields an orange solid (84 %).

Recently, another synthesis route of azide 14 was published.[3]

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.27.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 2924 (w), 2085 (s), 1593 (s), 1376 (w), 1170 (m).

^{1}H-NMR (400MHz; DMSO-d_6):

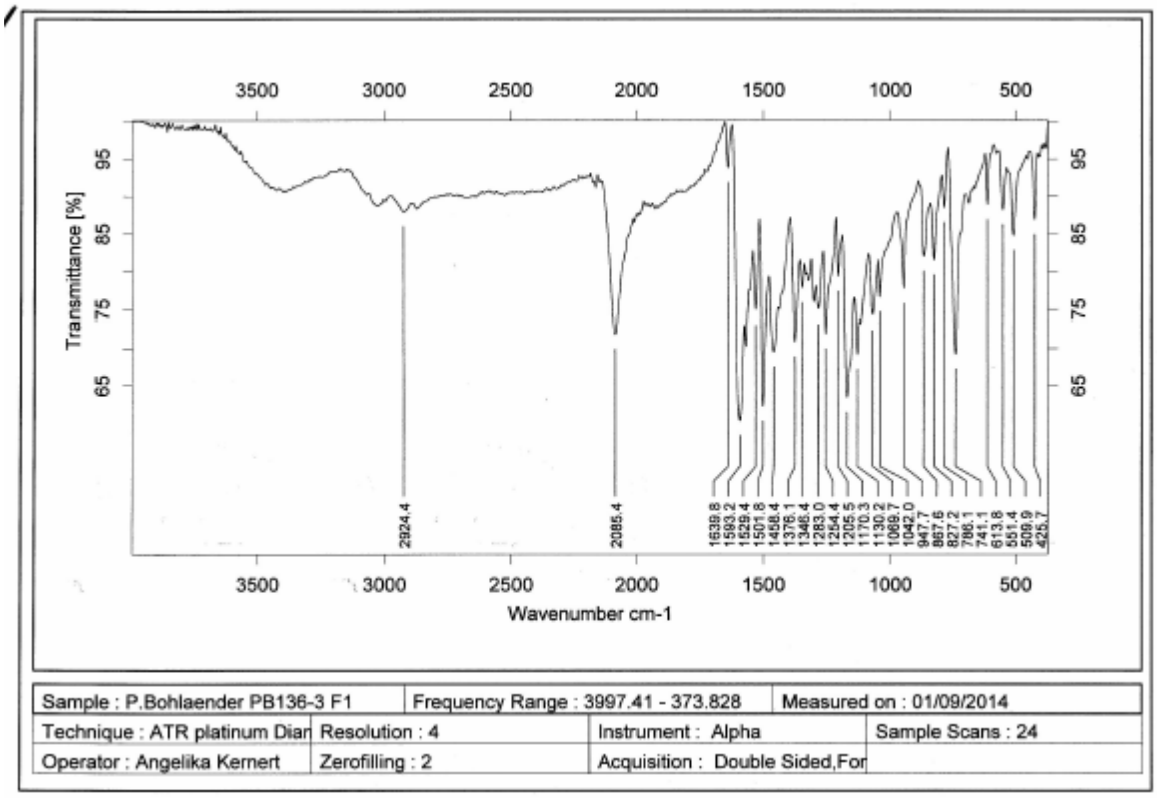
δ (ppm) = 2.18 (p, *J* = 6.7, 2H), 3.48 (t, *J* = 6.5, 2H), 3.89 (s, 3H), 4.48 (t, *J* = 7.1, 2H), 7.25 – 7.36 (m, 3H), 7.58 (d, *J* = 8.1, 1H), 7.97 (s, 1H), 8.13 – 8.27 (m, 4H), 8.76 (d, *J* = 6.7, 2H).

^{13}C-NMR (100 MHz, DMSO-d_6):

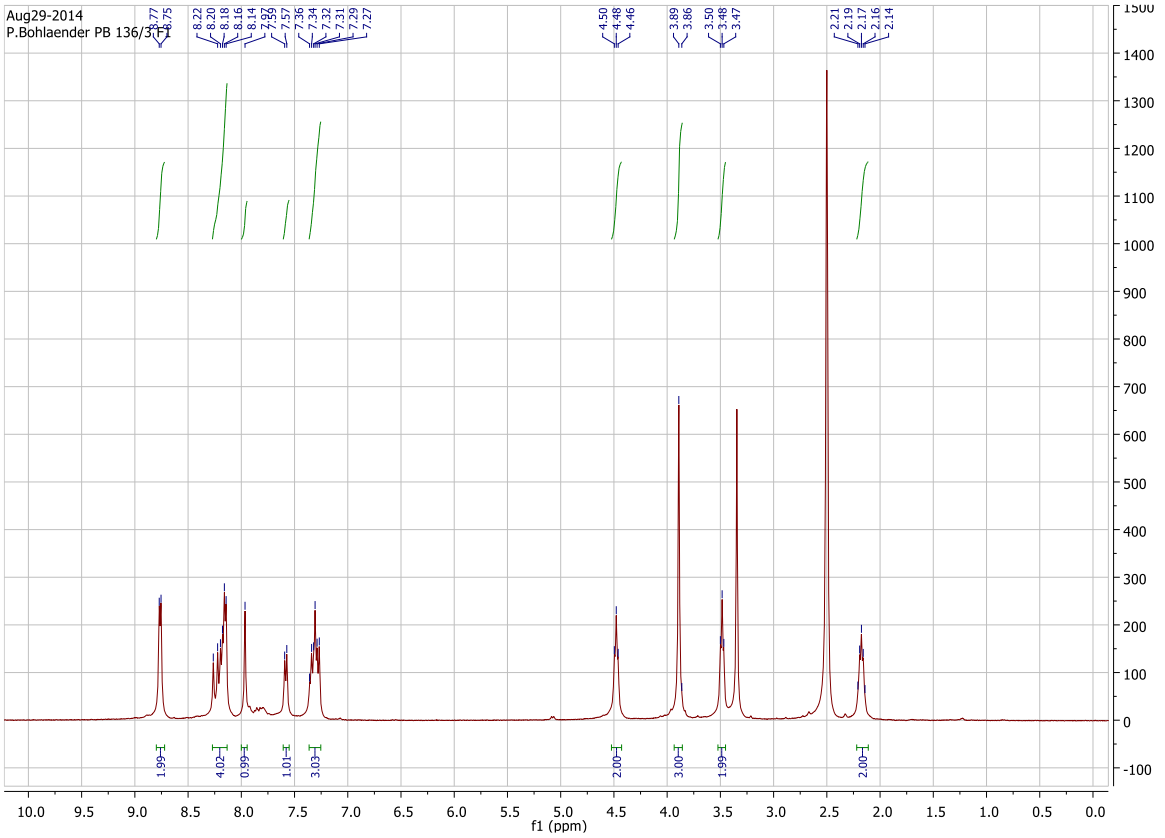
δ (ppm) = 29.5, 33.1, 47.6, 56.7, 111.0, 112.6, 116.8, 120.5, 121.4, 121.9, 123.0, 125.3, 135.8, 136.0, 138.0, 143.4, 154.6.

MS (FAB) m/z (%): 318.2 (100) [M^+].

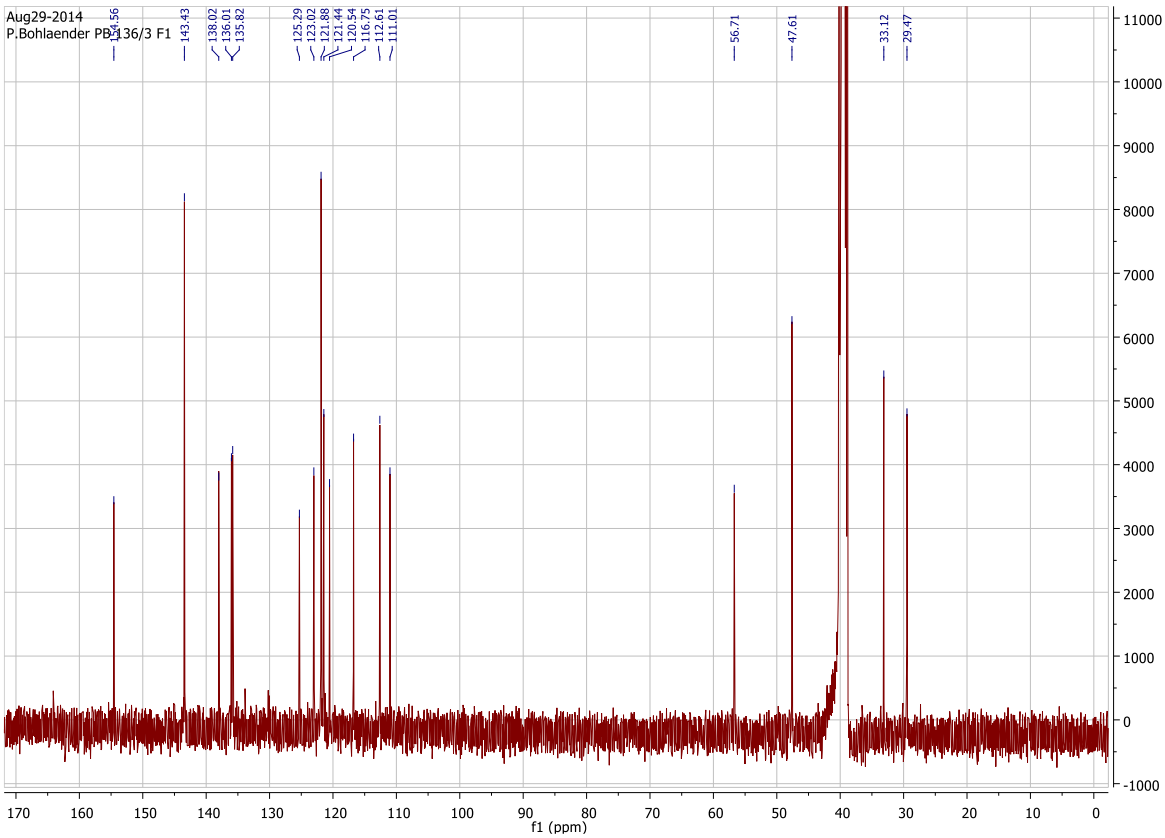
HR-MS (FAB) m/z: calculated for $C_{19}H_{20}N_5^+$ [M^+]: 318.1713, found: 318.1715.



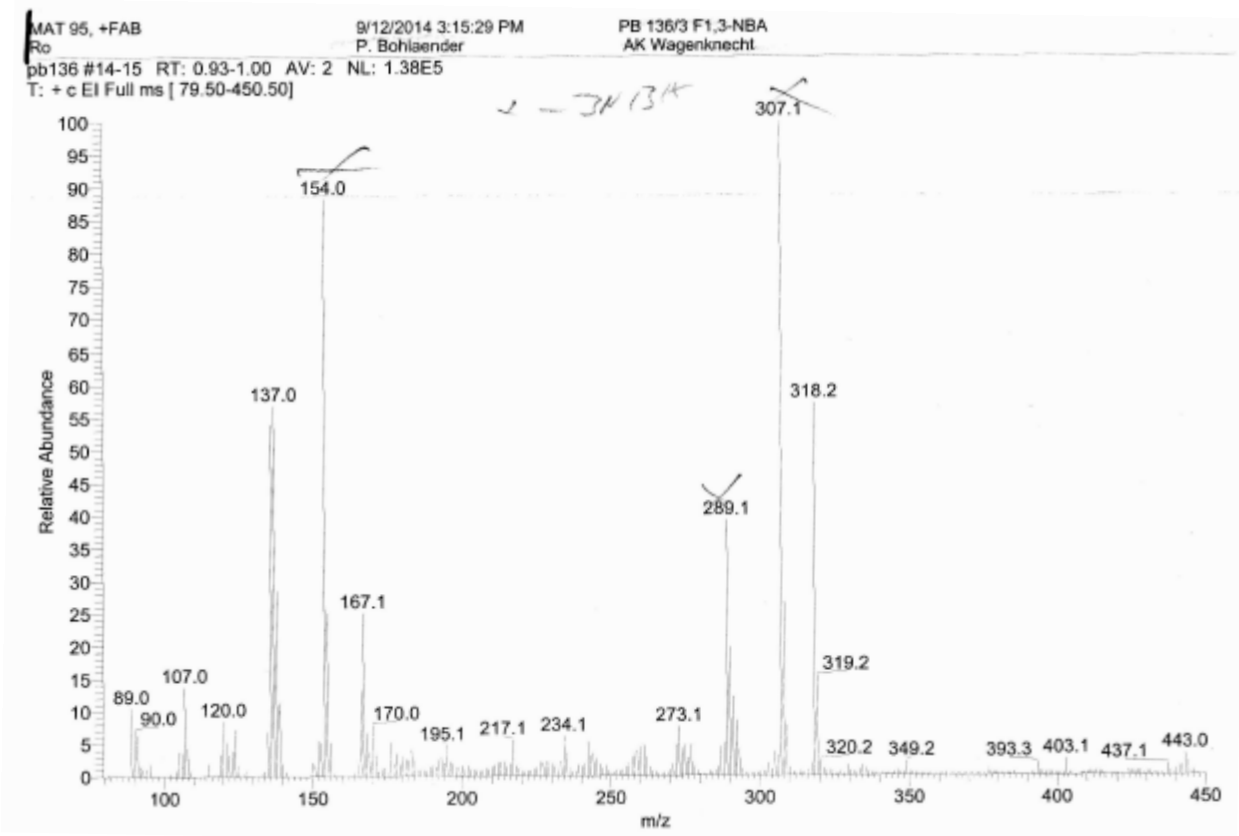
Scheme S46: IR of azide **14**.



Scheme S47: ^{1}H-NMR of azide **14**.



Scheme S48: ^{13}C-NMR of azide **14**.



Scheme S49: MS (FAB) of azide **14**.

pb136-c4#11 RT: 0.74
T: + c EI Full ms [79.48-450.48]
m/z= 318.0687-318.3179

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
318.1715	3062.0	100.00	318.1713	0.13	$C_{19}H_{20}N_5$

Scheme S50: HR-MS (FAB) of azide **14**.

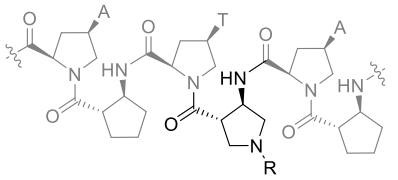
5. Strand displacement:

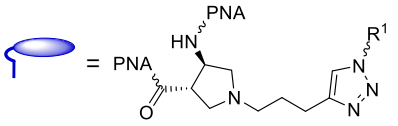
5.1 PNA and DNA sequences:

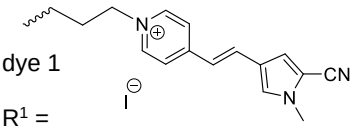
PNA strands:

PNA1 *C-* Lys_5 - ACG-AAT-ATTA-ACA-TC *-N*

PNA2 *C-* Lys_5 - ACG-AAT-ATTA-ACA-TC *-N*



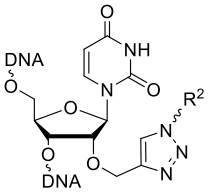




DNA strands:

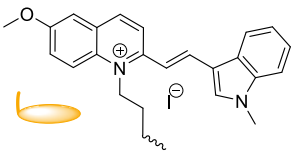
DNA1 5´- TA-TAT-TG - 3´

5´- C-TTA- -TGT - 3´



DNA2 UAT

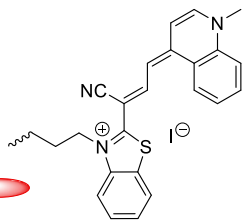
DNA4 TAU



5´- TGC-TTA- -TGT-AG - 3´

DNA3 UAT

DNA5 TAU



DNA6 3´- ACG-AAT-ATA-ACA-TC - 5

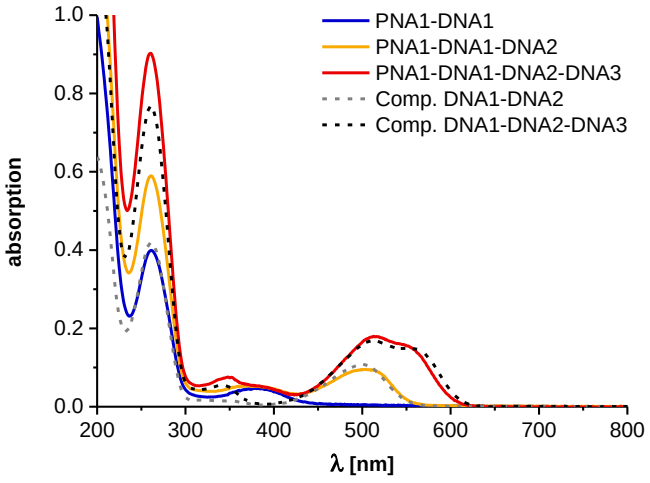
DNA11 5´- C-TTA-TAT-TGT - 3´

DNA12 5´- TGC-TTA-TAT-TGT-AG - 3´

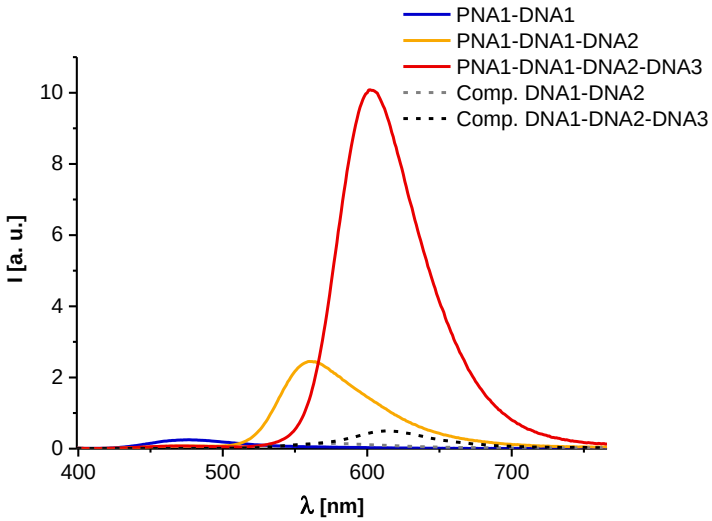
Scheme S51: PNA and DNA sequences of strand displacement.

5.2 Spectroscopic data:

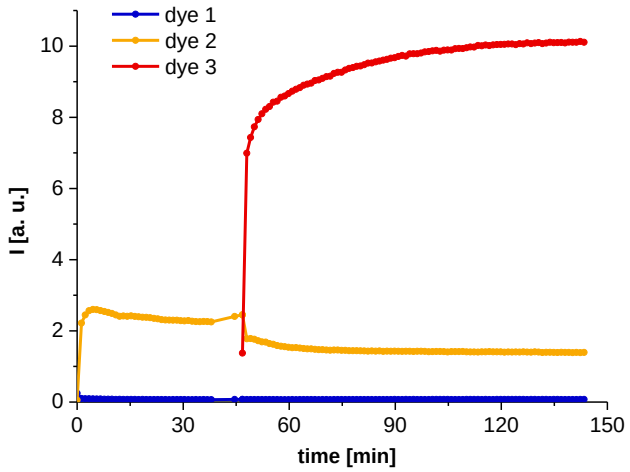
Spectroscopic measurements were recorded with 2.5 µM PNA/DNA in NaP_i buffer solution (10 mM, pH = 7) using quartz glass cuvettes (10 mm) at 20 °C. Absorption spectra were recorded with a Varian Cary 100 spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured with a Jobin–Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. Spectra were recorded with an excitation band pass and an emission band pass of 3 nm and were corrected for Raman emission from the buffer solution ($\lambda_{exc.}$ = 389 nm; $\lambda_{em.}$ = 399 – 766 nm). All strand displacement measurements were recorded after addition of 1.0 equivalent of each longer counterstrand to previously annealed PNA1 or PNA2 with DNA1, respectively. Thereby, the time to equilibrium determined in kinetic measurements was followed.



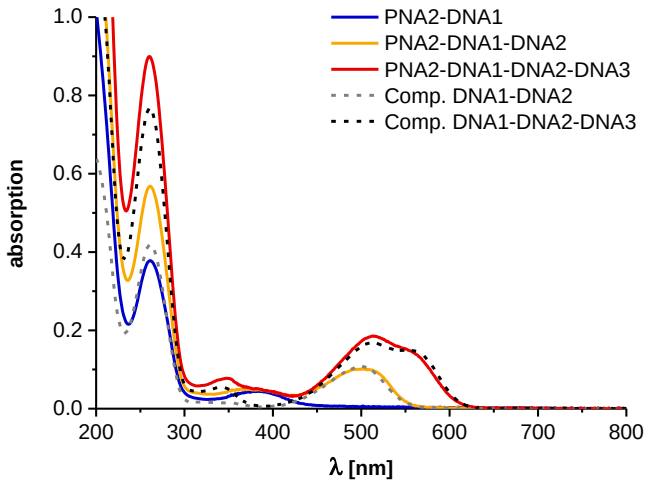
Scheme S52: Absorption spectra of PNA1-DNA1 before and after strand displacement with DNA2 and DNA3, respectively. Absorption spectra of comparison solutions containing DNA1-DNA2 and DNA1-DNA2-DNA3, respectively, are also displayed (dashed lines).



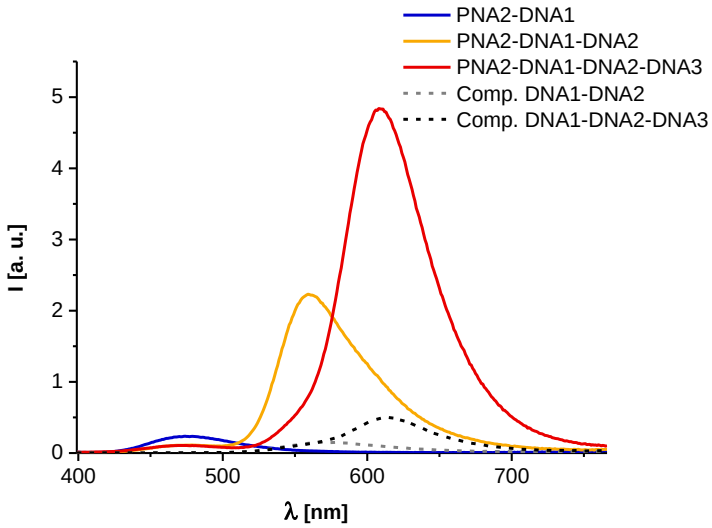
Scheme S53: Fluorescence spectra of PNA1-DNA1 before and after strand displacement with DNA2 and DNA3, respectively. Fluorescence spectra of comparison solutions containing DNA1-DNA2 and DNA1-DNA2-DNA3, respectively, are also displayed (dashed lines).



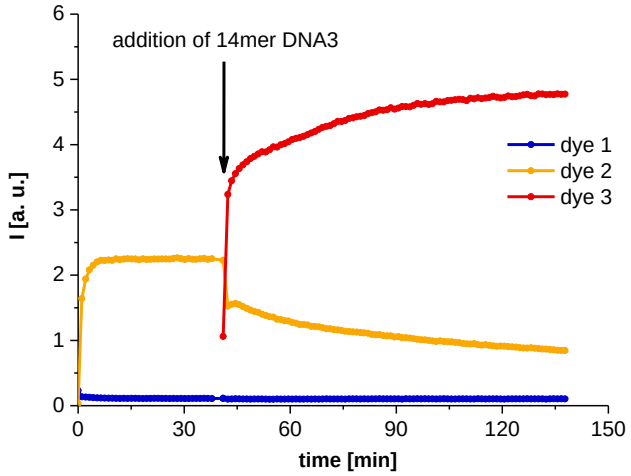
Scheme S54: Fluorescence intensity changes of dye 1 - 3 of strand displacement PNA1-DNA1 with DNA2 and DNA3, respectively. The atypical kinetic trend of the first strand displacement with DNA2 is explained in 5.2.1.



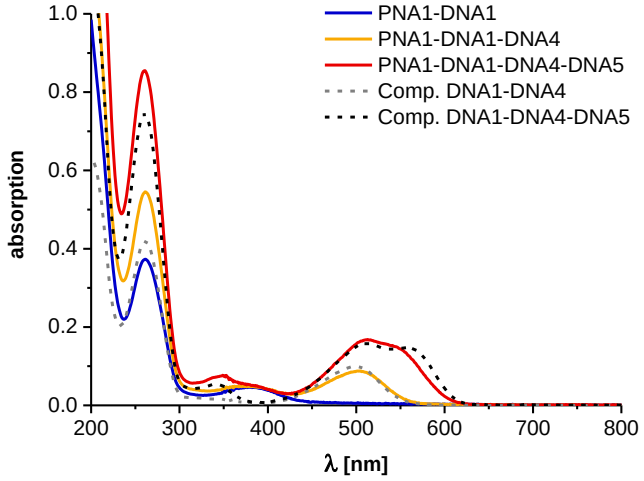
Scheme S55: Absorption spectra of PNA2-DNA1 before and after strand displacement with DNA2 and DNA3, respectively. Absorption spectra of comparison solutions containing DNA1-DNA2 and DNA1-DNA2-DNA3, respectively, are also displayed (dashed lines).



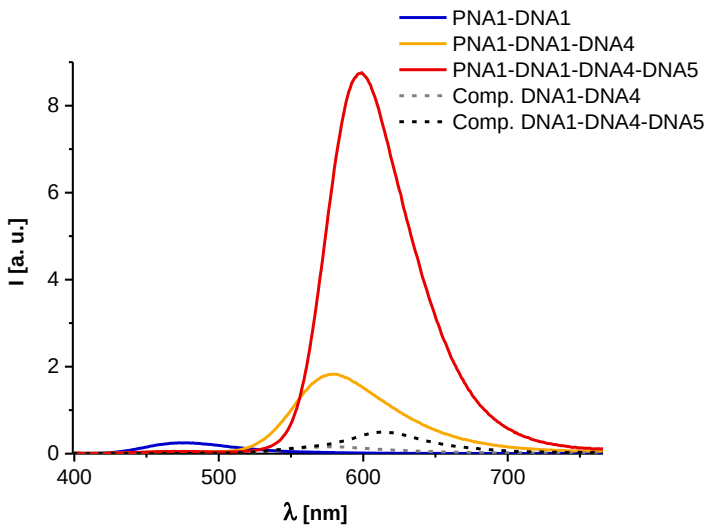
Scheme S56: Fluorescence spectra of PNA2-DNA1 before and after strand displacement with DNA2 and DNA3, respectively. Fluorescence spectra of comparison solutions containing DNA1-DNA2 and DNA1-DNA2-DNA3, respectively, are also displayed (dashed lines).



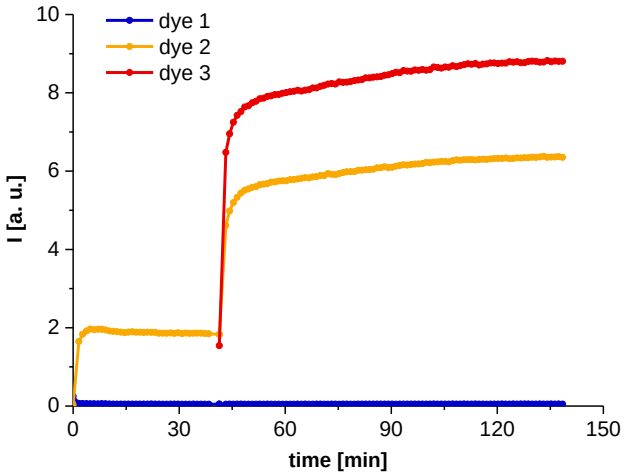
Scheme S57: Fluorescence intensity changes of dye 1 - 3 of strand displacement PNA2-DNA1 with DNA2 and DNA3, respectively.



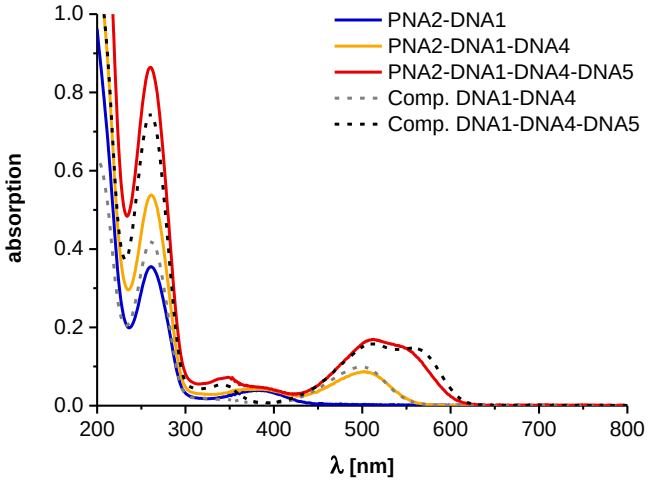
Scheme S58: Absorption spectra of PNA1-DNA1 before and after strand displacement with DNA4 and DNA5, respectively. Absorption spectra of comparison solutions containing DNA1-DNA4 and DNA1-DNA4-DNA5, respectively, are also displayed (dashed lines).



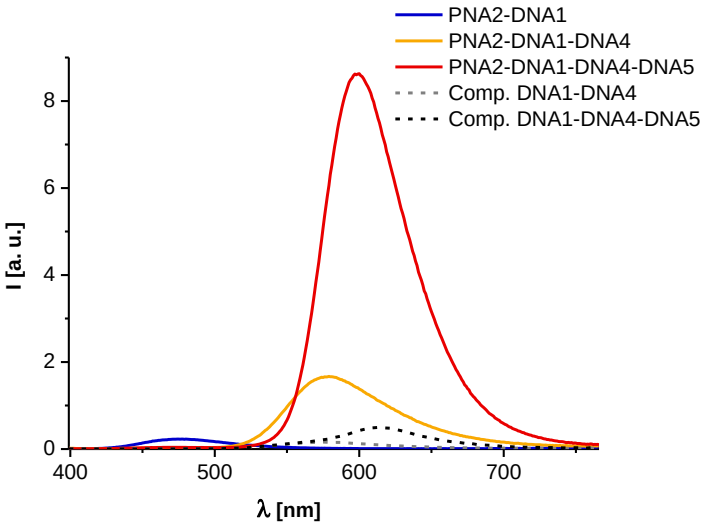
Scheme S59: Fluorescence spectra of PNA1-DNA1 before and after strand displacement with DNA4 and DNA5, respectively. Absorption spectra of comparison solutions containing DNA1-DNA4 and DNA1-DNA4-DNA5, respectively, are also displayed (dashed lines).



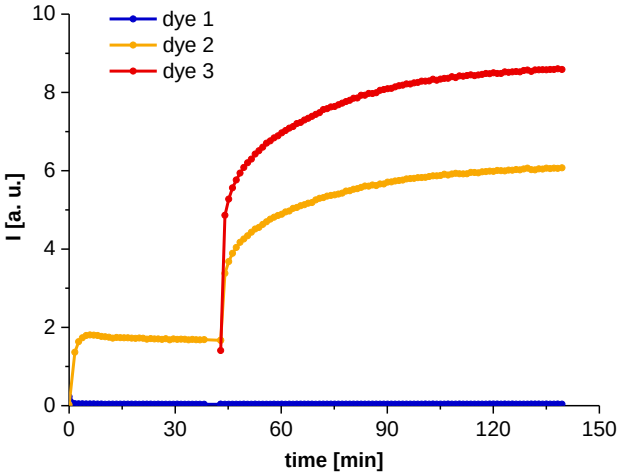
Scheme S60: Fluorescence intensity changes of dye 1 - 3 of strand displacement PNA1-DNA1 with DNA4 and DNA5, respectively.



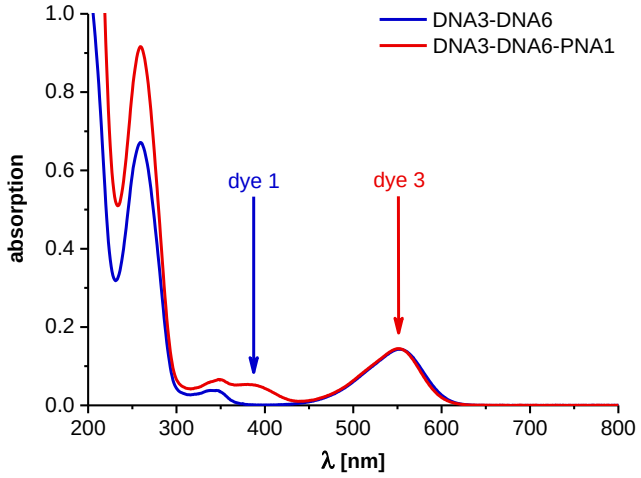
Scheme S61: Absorption spectra of PNA2-DNA1 before and after strand displacement with DNA4 and DNA5, respectively. Absorption spectra of comparison solutions containing DNA1-DNA4 and DNA1-DNA4-DNA5, respectively, are also displayed (dashed lines).



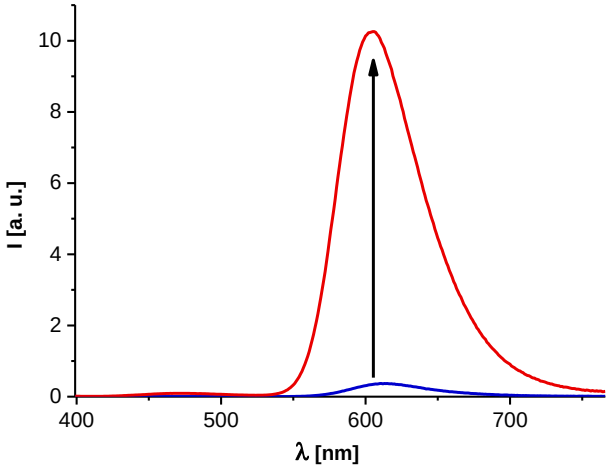
Scheme S62: Fluorescence spectra of PNA2-DNA1 before and after strand displacement with DNA4 and DNA5, respectively. Absorption spectra of comparison solutions containing DNA1-DNA4 and DNA1-DNA4-DNA5, respectively, are also displayed (dashed lines).



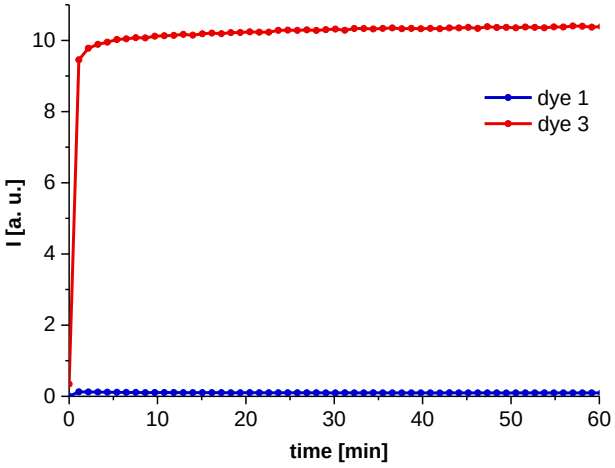
Scheme S63: Fluorescence intensity changes of dye 1 - 3 of strand displacement PNA2-DNA1 with DNA4 and DNA5, respectively.



Scheme S64: Absorption spectra of DNA3-DNA6 before and after strand displacement with PNA1.



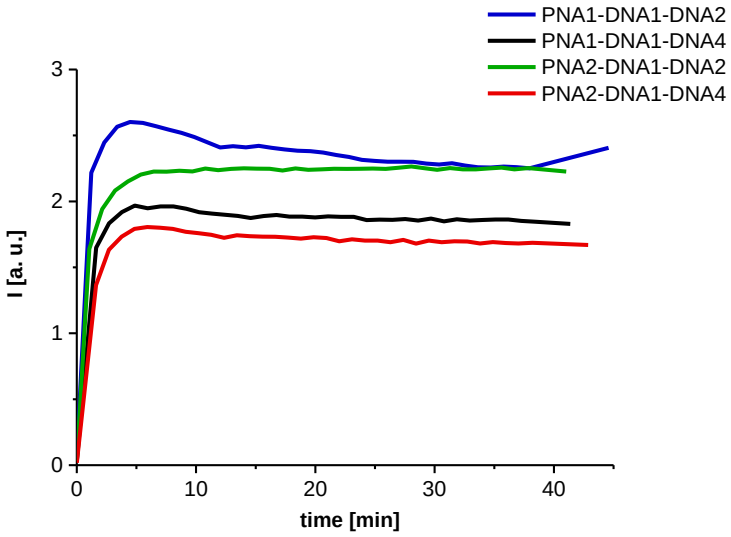
Scheme S65: Fluorescence spectra of DNA3-DNA6 before and after strand displacement with PNA1.



Scheme S66: Fluorescence intensity changes of dye 1 and dye 3 of strand displacement DNA3-DNA6 with PNA1.

5.2.1 Comment to kinetic trend:

Kinetic measurements of the first strand displacement PNA1-DNA1 and PNA2-DNA1 with DNA2 and DNA4, respectively exhibit unexpected but reproducible emission intensity changes. All kinetic trends are compared in the following scheme.



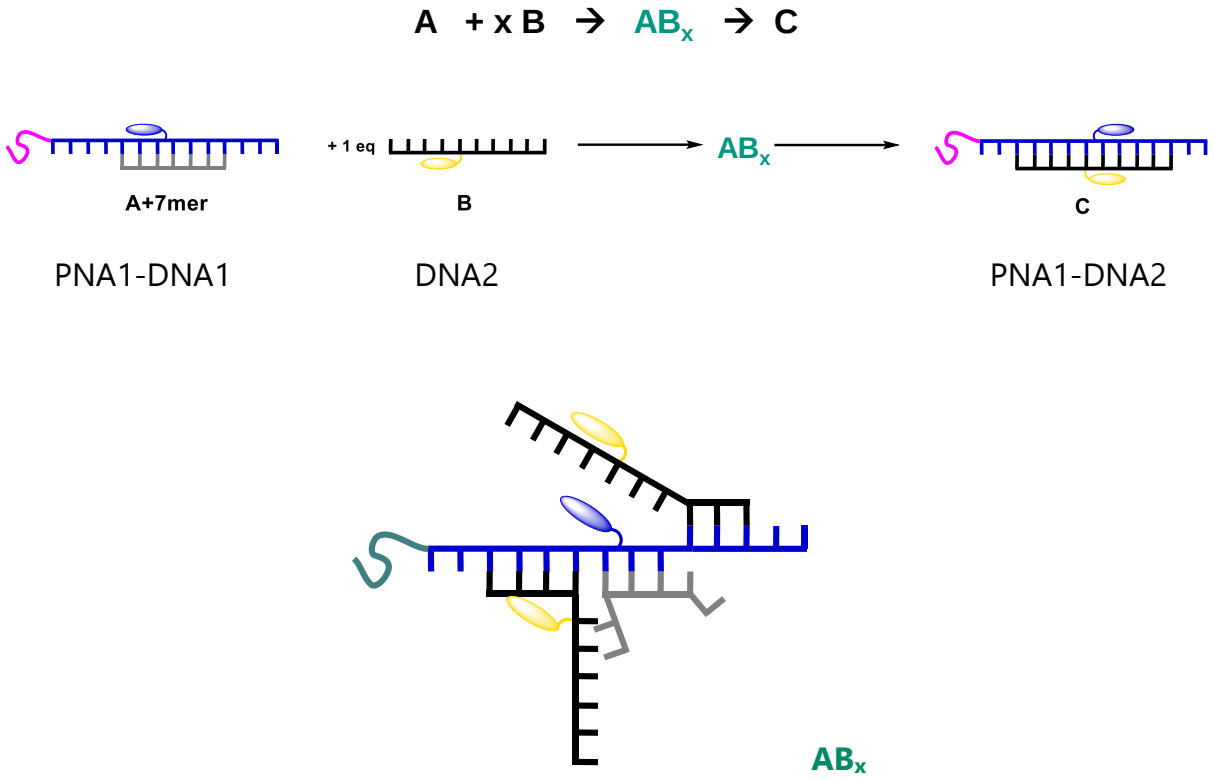
Scheme S67: Comparison of all fluorescence intensity changes of dye 2 in course of the first strand displacement with DNA2 and DNA4, respectively.

Only sample PNA2-DNA1-DNA2 shows a "typical" kinetic trend, whereas the other three samples and to the greatest extend sample PNA1-DNA1-DNA2 have rather atypical fluorescence intensity changes.

A plausible explanation can be assumed by comparison to the literature.[4] *Nielsen* observed an almost similar trend of CD-spectra recorded during triplex invasion of aegPNA into double-stranded DNA. *Nielsen* supposed the formation of an intermediate with more than one PNA-strand.

Regarding to the literature it is most likely that the observed kinetic trends reflect the formation of an intermediate with more than one DNA-strand (AB_x, with A = PNA and B = dye 2-modified DNA). The initial increase of fluorescence intensity might be an effect of a temporary attachment of two acceptor-modified DNA strands to the PNA strand that carries the donor dye 1. If both acceptor dyes 2 are in optimal proximity to the donor dye 1 an energy transfer can occur to both acceptor dyes, respectively. In

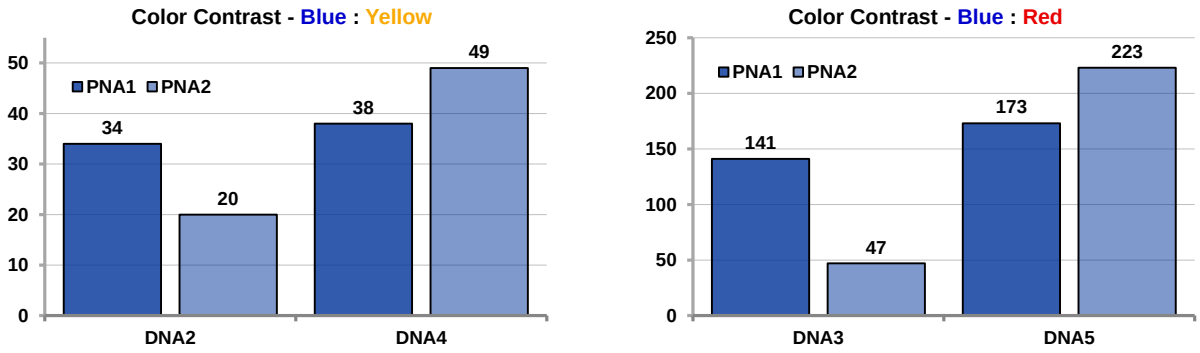
this case, the initial emission intensity is higher than the emission intensity of the final conformation (C).



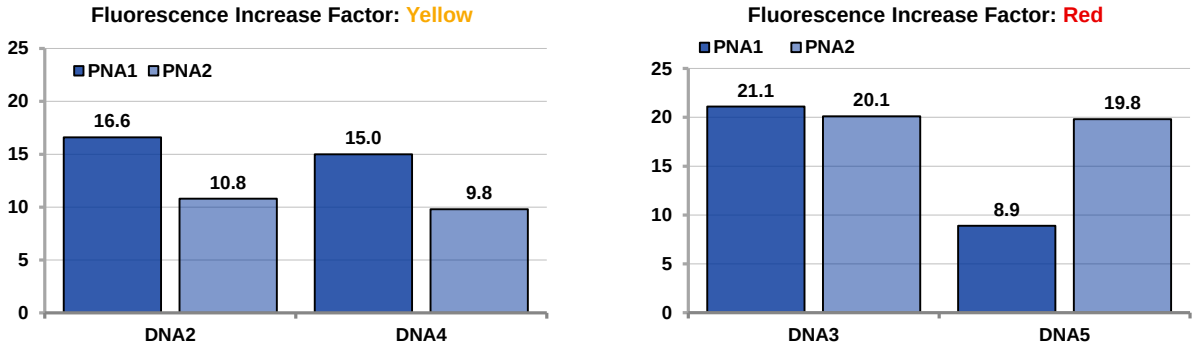
Scheme S68: Assumed formation of the most likely generated intermediate ABx before the final conformation (C) is formed.

5.3 Color contrasts and fluorescence intensity increase factors:

Fluorescence intensity increase factors were calculated by determined fluorescence intensity enhancement from each value of comparison solution to final fluorescence intensity of sample solution, respectively.



Scheme S69: Fluorescence color contrasts of strand displacement. Color contrasts blue:yellow (left) and blue:red (right).



Scheme S70: Fluorescence intensity increase factors of strand displacement. Increase factors yellow (left) and red (right).

5.4 ET-efficiencies and fluorescence lifetimes:

Fluorescence lifetimes were recorded of sample solutions containing 2.5 µM PNA/DNA in NaP_i buffer solution (10 mM, pH = 7) using quartz glass cuvettes (10 mm) at 20 °C. Fluorescence lifetime measurement was performed with Horiba Scientific FluoroMax-4 spectrofluorometer using a time-correlated single photon counting (TCSPC) technique with excitation source NanoLed at 370 nm (Horiba, impulse repetition rate of 1 MHz, time calibration = 2.74E-11 sec/ch) and emission was detected at 478 nm. Lifetimes were calculated with DAS6 v 6.8 decay analysis software (Horiba), mono-exponential fit for donor lifetimes in absence of acceptor dye and bi- exponential fit for donor lifetimes in presence of acceptor dye.

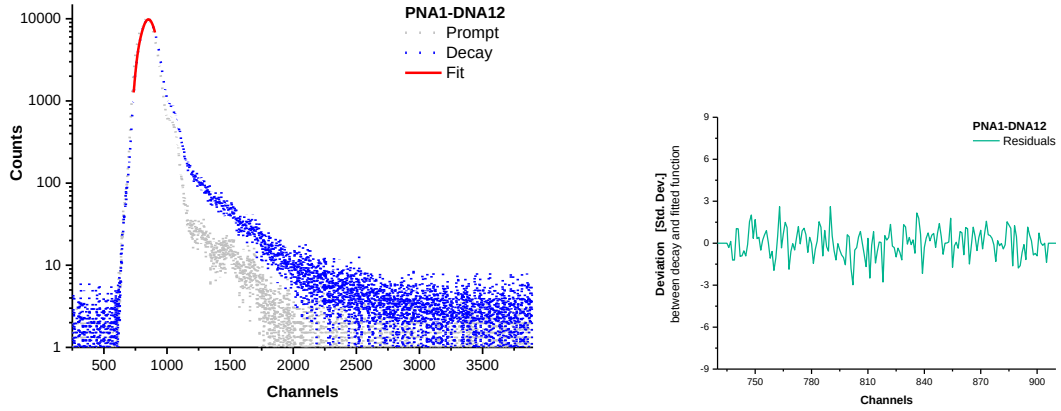
Energy transfer efficiencies were calculated by following equation:

$$E_{transfer} = 1 - \frac{\tau_{DA}}{\tau_D}$$

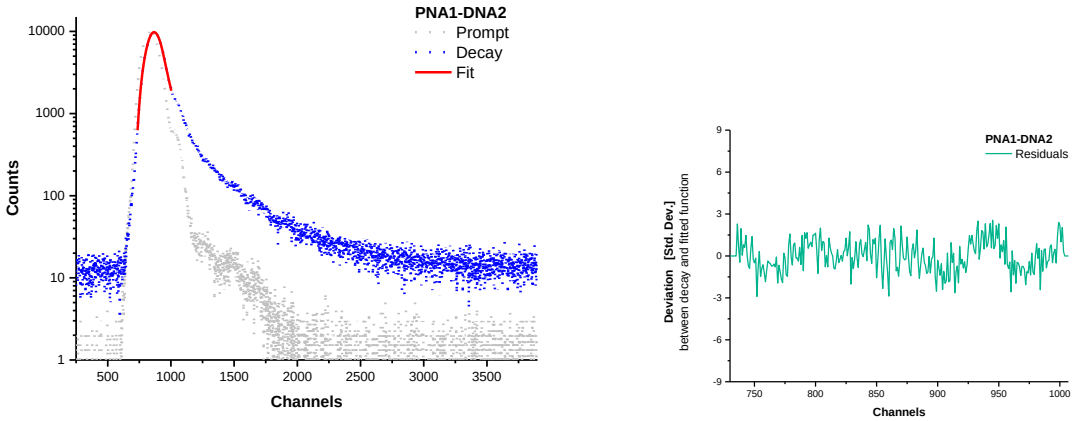
With: τ_{DA} = lifetime of donor dye 1 (τ_{dye1}) in presence of acceptor dye 2 or dye 3

τ_D = lifetime of donor dye 1 (τ_{dye1}) in absence of acceptor dye

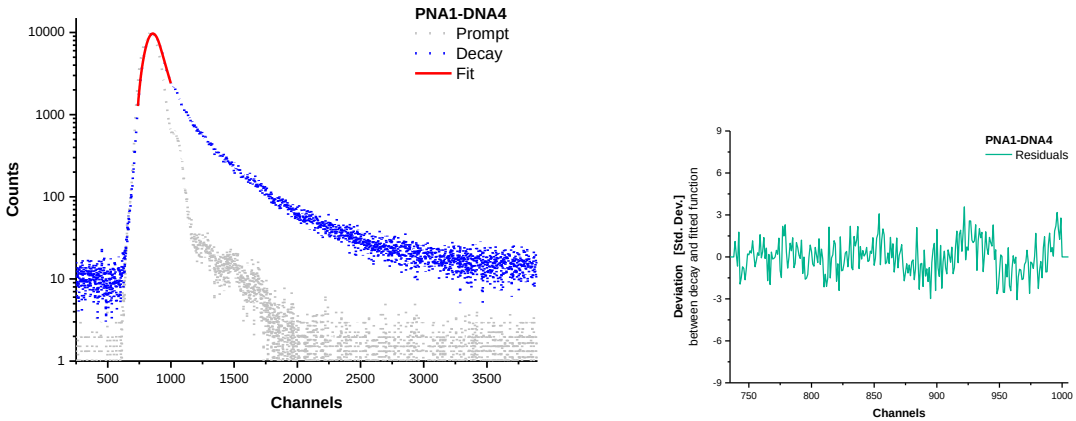
τ_{dye1}-Decay graphs:



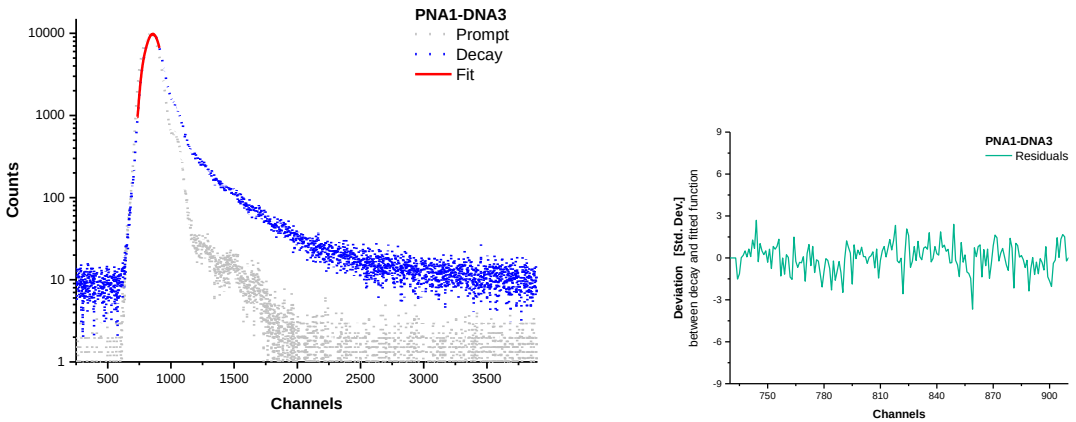
Scheme S71: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA1-DNA12.



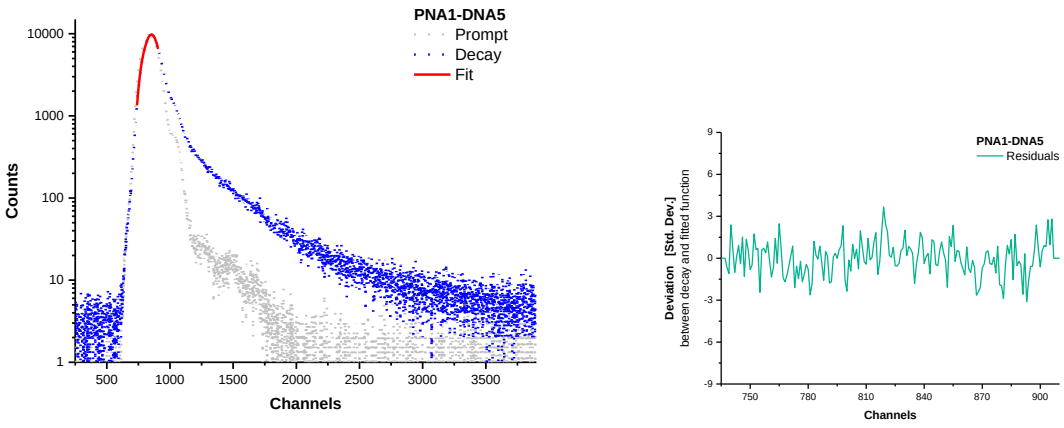
Scheme S72: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA1-DNA2.



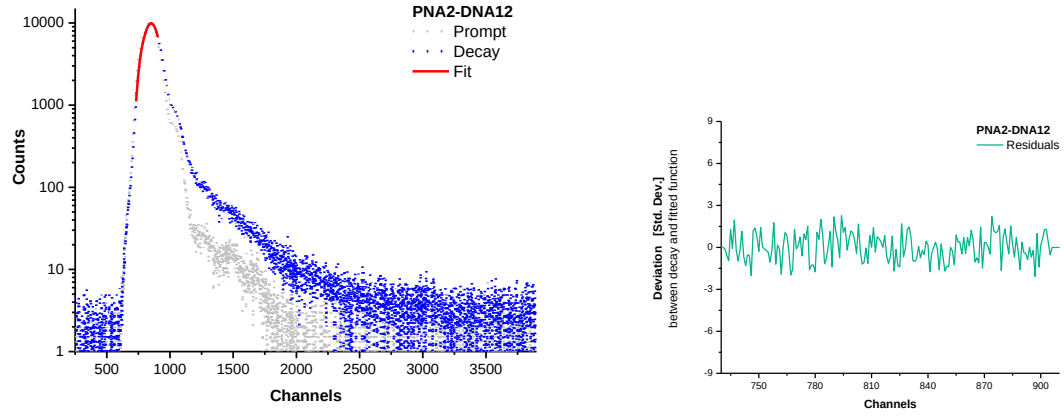
Scheme S73: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA1-DNA4.



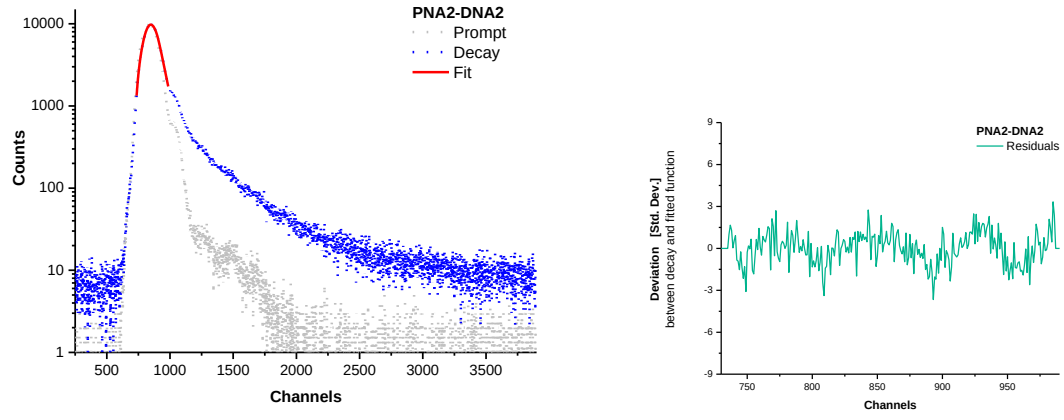
Scheme S74: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA1-DNA3.



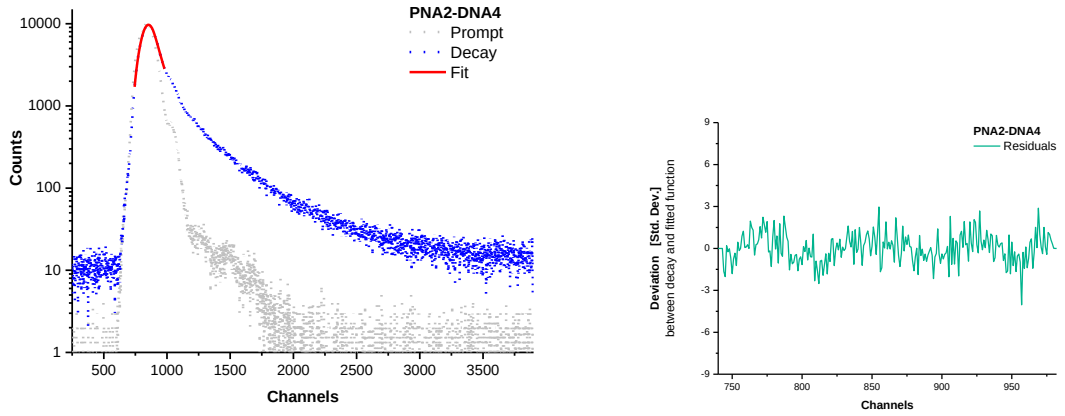
Scheme S75: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA1-DNA5.



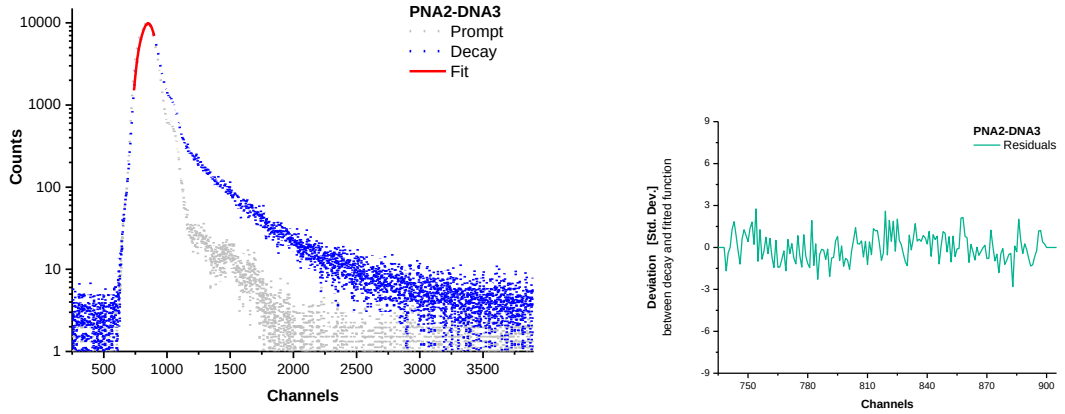
Scheme S76: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA2-DNA12.



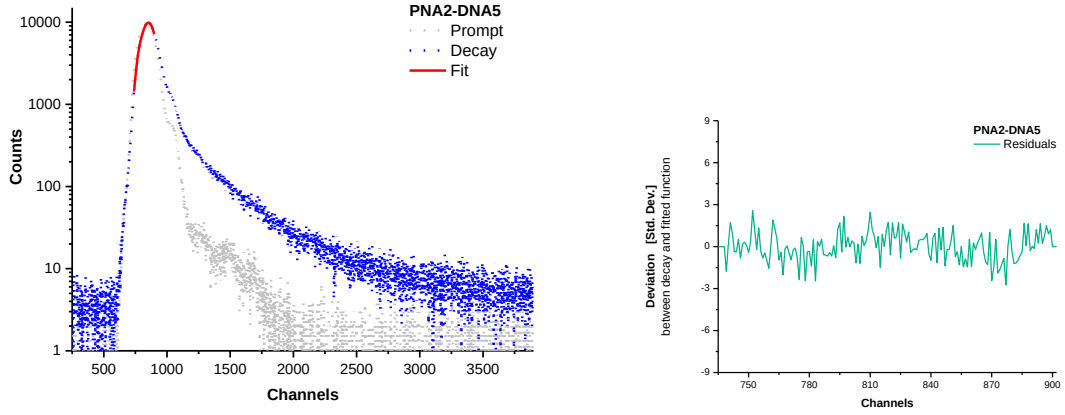
Scheme S77: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA2-DNA2.



Scheme S78: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA2-DNA4.



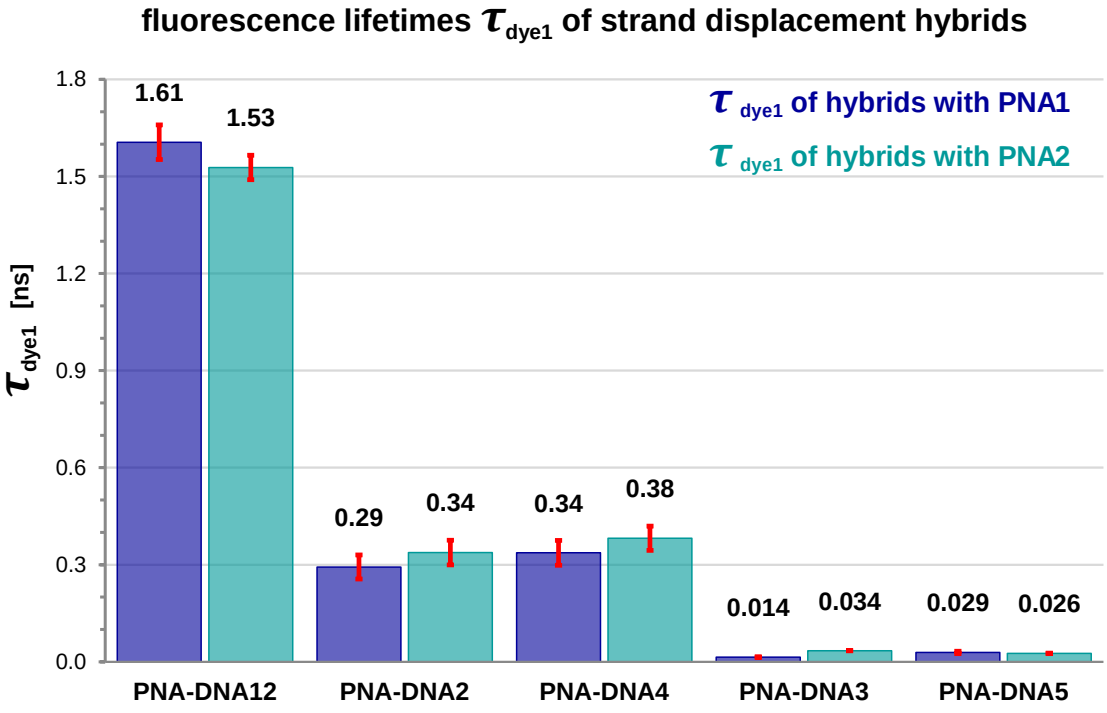
Scheme S79: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA2-DNA3.



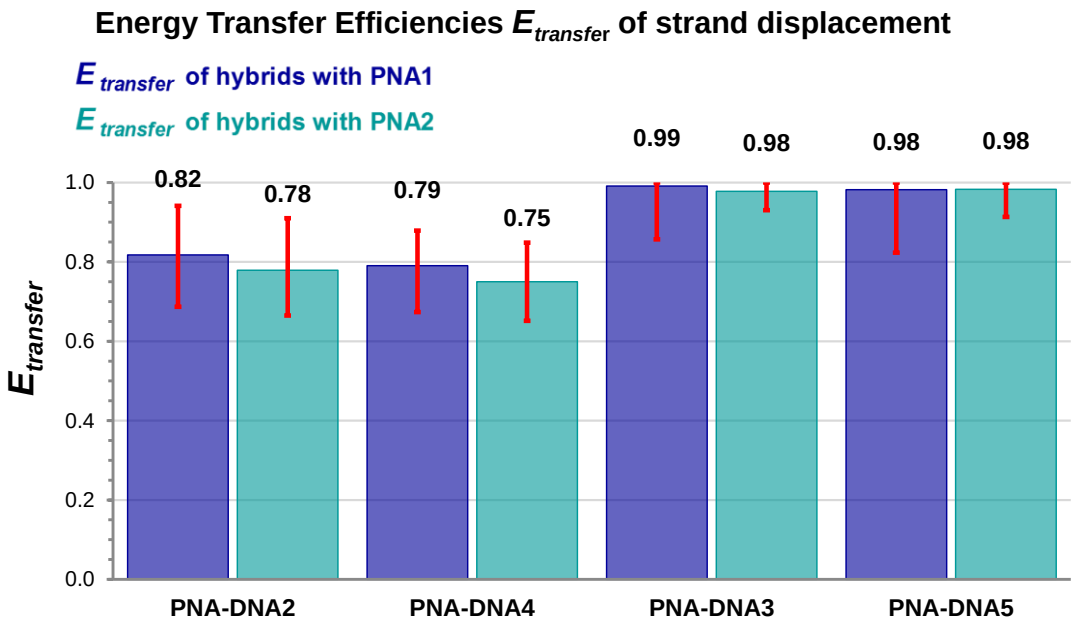
Scheme S80: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand displacement hybrid PNA2-DNA5.

Duplex	τ_{dye1} [ns]	$\Delta\tau_{dye1}$ [ns]	$\Delta\tau_{dye1}$ [%]	$E_{transfer}$	$\Delta E_{transfer}$ [%]	$\Delta E_{transfer}$
PNA1-DNA12	1.606	0.0536	3.34	-	-	-
PNA1-DNA2	0.293	0.0371	12.65	0.818	16.0	0.131
PNA1-DNA4	0.337	0.0385	11.44	0.790	14.8	0.117
PNA1-DNA3	0.014	0.0014	10.22	0.991	13.6	0.134
PNA1-DNA5	0.029	0.0037	12.84	0.982	16.2	0.159
PNA2-DNA12	1.528	0.0377	2.47	-	-	-
PNA2-DNA2	0.337	0.0382	11.32	0.779	14.7	0.114
PNA2-DNA4	0.382	0.0375	9.81	0.750	13.1	0.099
PNA2-DNA3	0.034	0.0005	1.50	0.978	4.8	0.047
PNA2-DNA5	0.026	0.0010	3.77	0.983	7.1	0.070

Table S1: Fluorescence lifetimes and energy transfer efficiencies of strand displacement hybrids.



Scheme S81: Fluorescence lifetimes of donor dye 1 of strand displacement hybrids.



Scheme S82: Energy transfer efficiencies of strand displacement hybrids.

5.5 Melting temperatures:

λ = 260 nm; 5 - 95 °C; interval: 0.5 °C/min; 2.5 µM PNA/DNA in 10 mM NaP_i-buffer (pH = 7.0). For comparison melting temperatures of DNA/DNA homodimers were additionally determined in presence of 250 mM NaCl.

duplex (1:1)	mp [°C]	
	without NaCl	with 250 mM NaCl
PNA1-DNA1	71.0	-
PNA2-DNA1	71.5	-
DNA6-DNA1	< 10	10.1
PNA1-DNA11	> 90	-
PNA2-DNA11	> 90	-
DNA6-DNA11	< 10	31.4
PNA1-DNA2	78.2	-
PNA2-DNA2	77.2	-
DNA6-DNA2	< 20	35.1
PNA1-DNA4	> 90	-
PNA2-DNA4	82.7	-
DNA6-DNA4	< 20	35.1
PNA1-DNA12	> 90	-
PNA2-DNA12	> 90	-
DNA6-DNA12	28.9	48.9
PNA1-DNA3	> 90	
PNA2-DNA3	> 90	
DNA6-DNA3	32.7	51.1
PNA1-DNA5	> 90	-
PNA2-DNA5	> 90	-
DNA6-DNA5	32.7	51.4

Table S2: Melting temperatures of strand displacement.

6. Strand invasion:

6.1 PNA and DNA sequences:

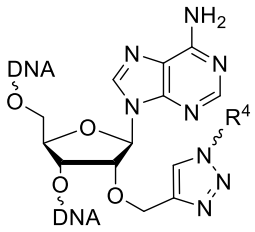
dsDNA strands:

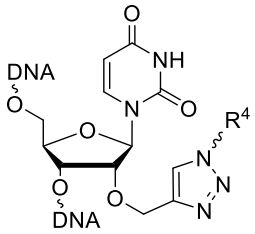
dsDNA 3´- GGC-TCC-TT-AA-TAT-ATA-AAG-AAA-TT-CCT-CGG - 5´

5´- CCG-AGG-AA-TT-ATA-[ ]-TTC-TTT-AA-GGA-GCC - 3´

dsDNA7 TAT R^4 = dye 1 **dsDNA8** TAU R^4 = dye 1

dsDNA9 TAT R^4 = dye 3 **dsDNA10** TAU R^4 = dye 3

A = 

U = 

dsDNA13 3´- GGC-TCC-TT - 5´

5´- CCG-AGG-AA - 3´

ssDNA strands:

DNA14 3´- AA-TAT-ATA-AAG-AAA - 5´

DNA15 3´- GGC-TCC-TT-AA-TAT-ATA-AAG-AAA-TT-CCT-CGG - 5´

DNA 5´- CCG-AGG-AA-TT-ATA-[ ]-TTC-TTT-AA-GGA-GCC - 3´

DNA16 TAT

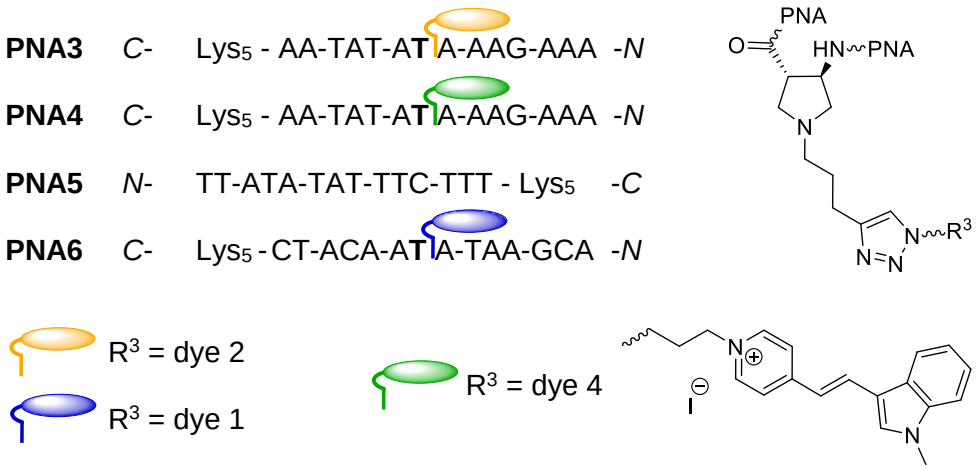
DNA17 TAT R^4 = dye 1 **DNA18** TAU R^4 = dye 1

DNA19 TAT R^4 = dye 3 **DNA20** TAU R^4 = dye 3

DNA21 5´- TT-ATA-TAT-TTC-TTT - 3´

Scheme S83: DNA sequences of strand invasion.

PNA strands:

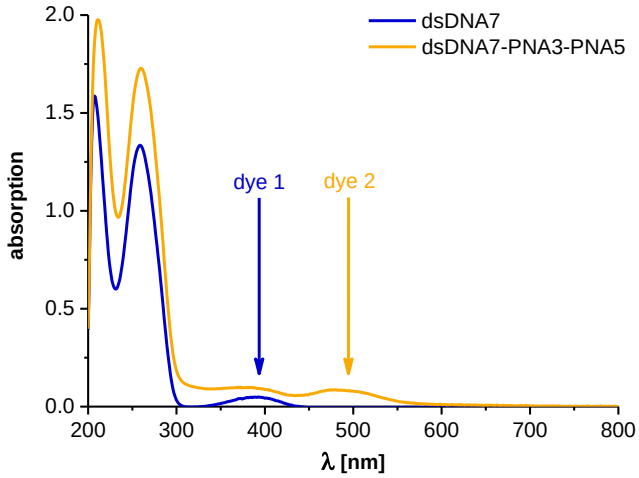


Scheme S84: PNA sequences of strand invasion.

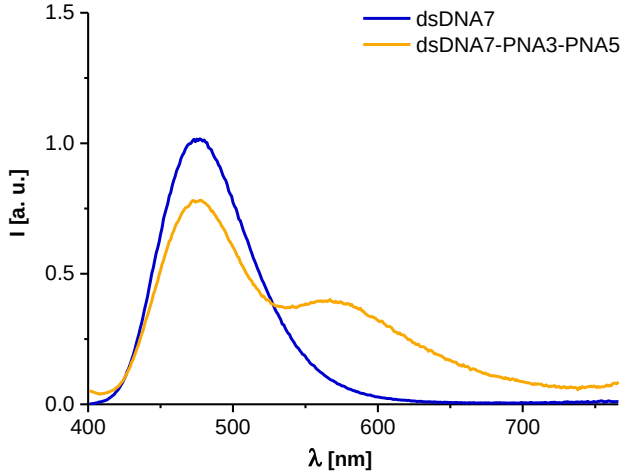
6.2 Spectroscopic data:

Spectroscopic measurements were recorded with 2.5 µM PNA/DNA in NaP_i buffer solution (10 mM, pH = 7) in absence and presence of 250 mM NaCl, respectively, using quartz glass cuvettes (10 mm) at 20 °C. Absorption spectra were recorded with a Varian Cary 100 spectrometer equipped with a 6x6 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. Spectra were recorded with an excitation band pass and an emission band pass of 3 nm (if not explicit denoted below the spectrum) and were corrected for Raman emission from the buffer solution (donor dye 1: $\lambda_{exc.}$ = 391 nm; $\lambda_{em.}$ = 401 – 766 nm, donor dye 4: $\lambda_{exc.}$ = 410 nm; $\lambda_{em.}$ = 425 – 800 nm). Strand invasion measurements were recorded after addition of 3.0 equivalents (kinetic: 1.0 equivalent, titration: until 3.0 equivalents) of PNA3-PNA5 or 1.5 equivalents (kinetic: 1.0 equivalent, titration: until 2.5 equivalents) PNA4-PNA5 e.g. 1.5 equivalents PNA6, to previously annealed dsDNA7 - 10, respectively. Thereby, the time to equilibrium determined in kinetic measurements was followed.

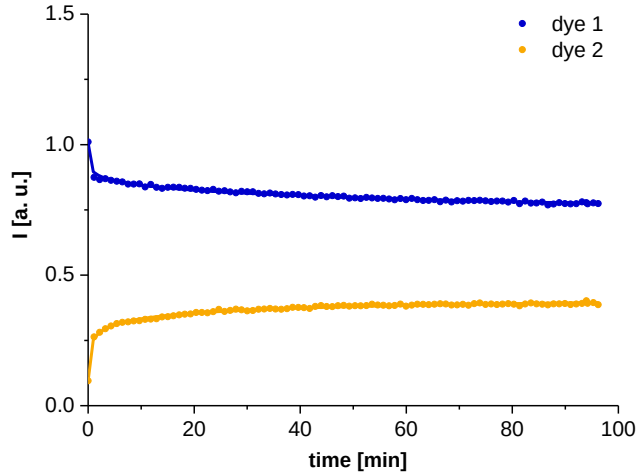
6.2.1 Spectroscopic data of kinetic measurements:



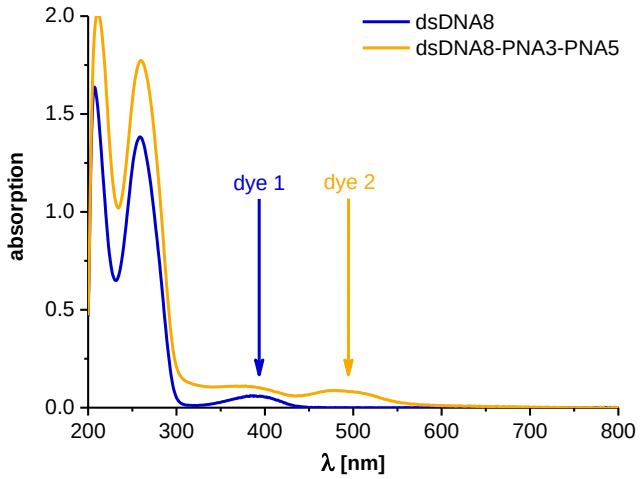
Scheme S85: Absorption spectra of strand invasion kinetic of dsDNA7-PNA3-PNA5 in presence of NaCl.



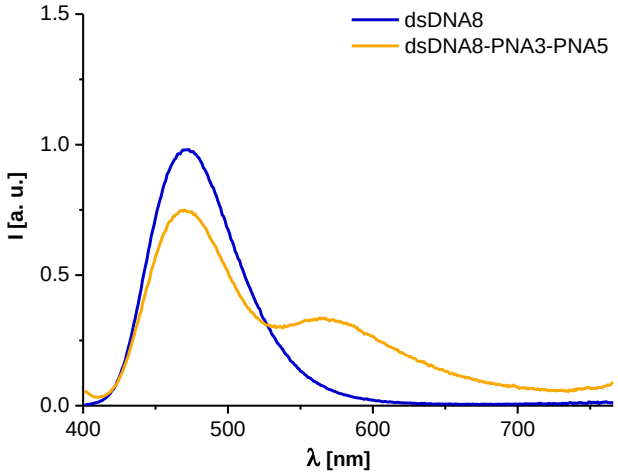
Scheme S86: Fluorescence spectra of strand invasion kinetic of dsDNA7-PNA3-PNA5 in presence of NaCl.



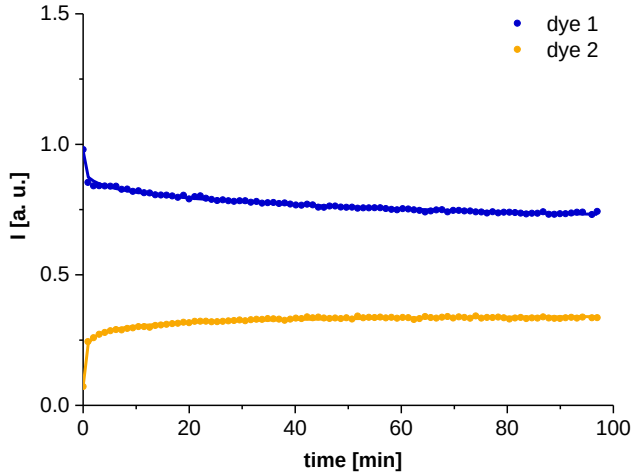
Scheme S87: Fluorescence intensity changes of dye 1 and dye 2 of strand invasion kinetic of dsDNA7-PNA3-PNA5 in presence of NaCl.



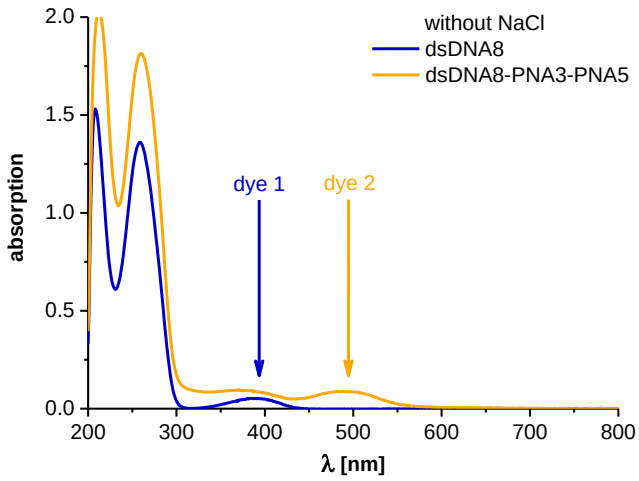
Scheme S88: Absorption spectra of strand invasion kinetic of dsDNA8-PNA3-PNA5 in presence of NaCl.



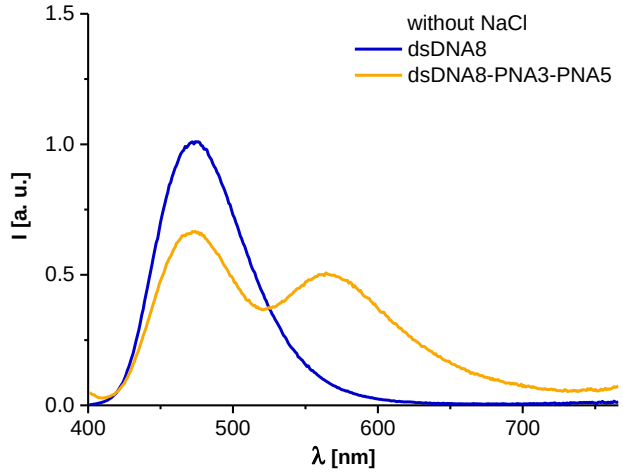
Scheme S89: Fluorescence spectra of strand invasion kinetic of dsDNA8-PNA3-PNA5 in presence of NaCl.



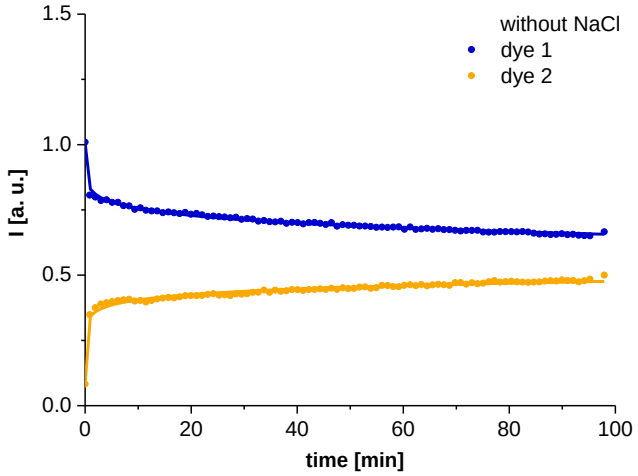
Scheme S90: Fluorescence intensity changes of dye 1 and dye 2 of strand invasion kinetic of dsDNA8-PNA3-PNA5 in presence of NaCl.



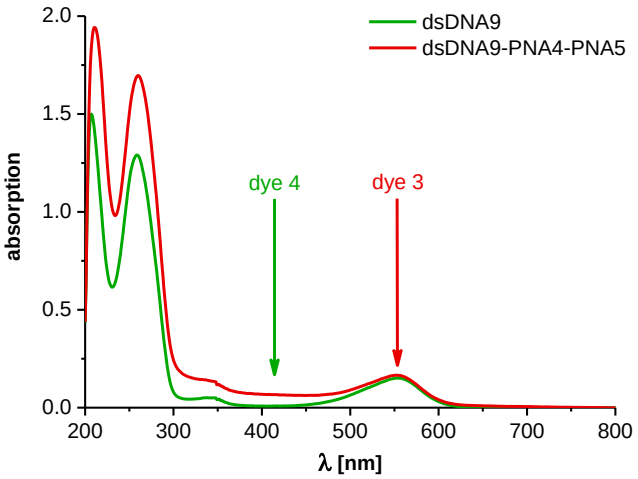
Scheme S91: Absorption spectra of strand invasion kinetic of dsDNA8-PNA3-PNA5 in absence of NaCl.



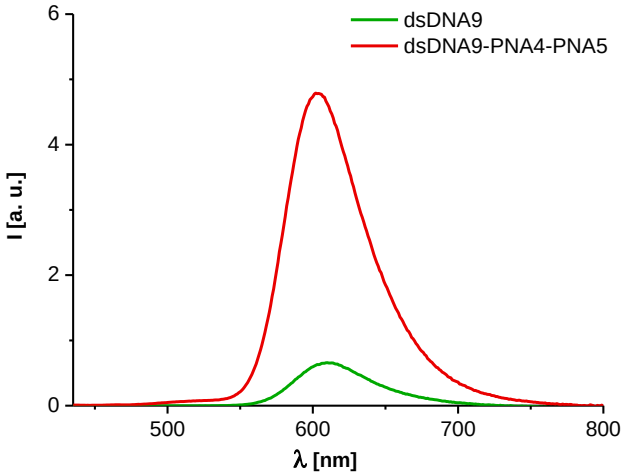
Scheme S92: Fluorescence spectra of strand invasion kinetic of dsDNA8-PNA3-PNA5 in absence of NaCl.



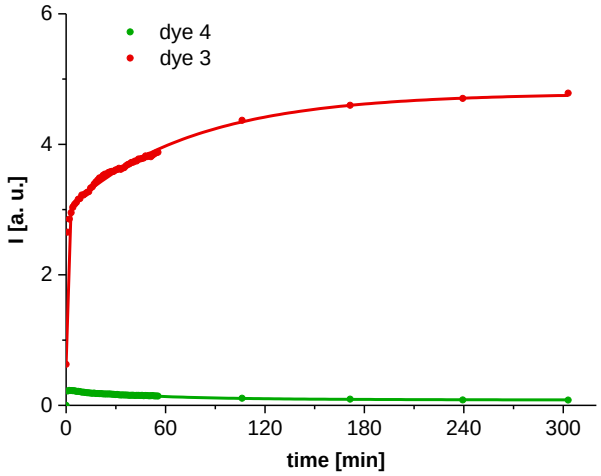
Scheme S93: Fluorescence intensity changes of dye 1 and dye 2 of strand invasion kinetic of dsDNA8-PNA3-PNA5 in absence of NaCl.



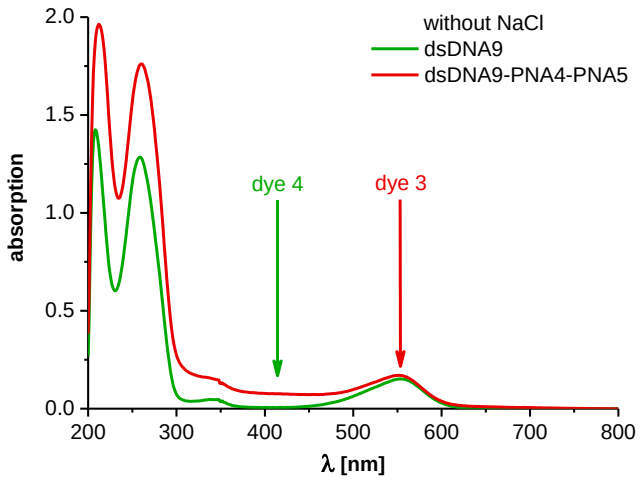
Scheme S94: Absorption spectra of strand invasion kinetic of dsDNA9-PNA4-PNA5 in presence of NaCl.



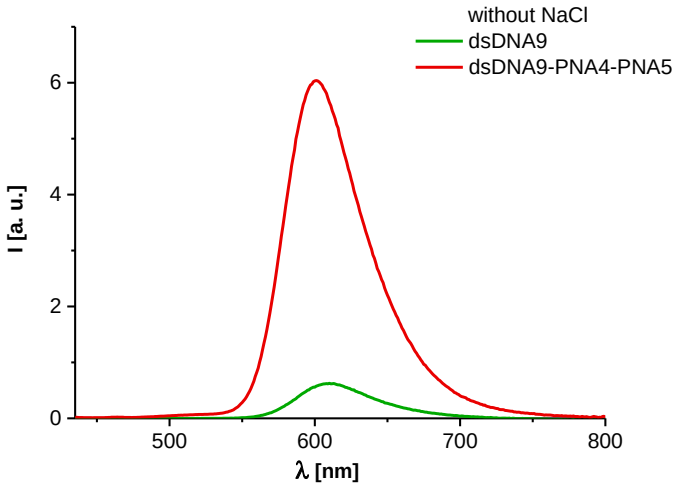
Scheme S95: Fluorescence spectra of strand invasion kinetic of dsDNA9-PNA4-PNA5 in presence of NaCl.



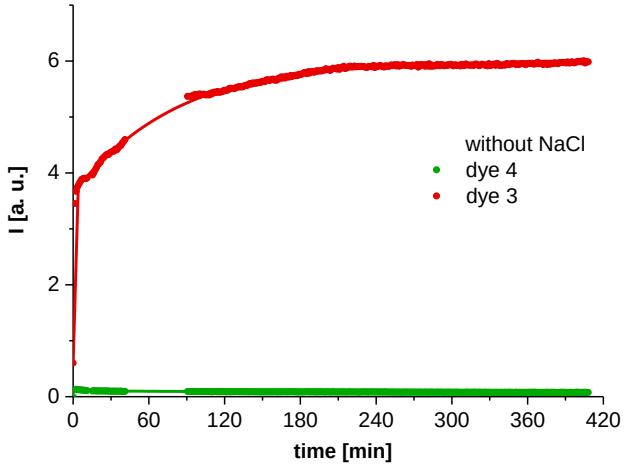
Scheme S96: Fluorescence intensity changes of dye 4 and dye 3 of strand invasion kinetic of dsDNA9-PNA4-PNA5 in presence of NaCl.



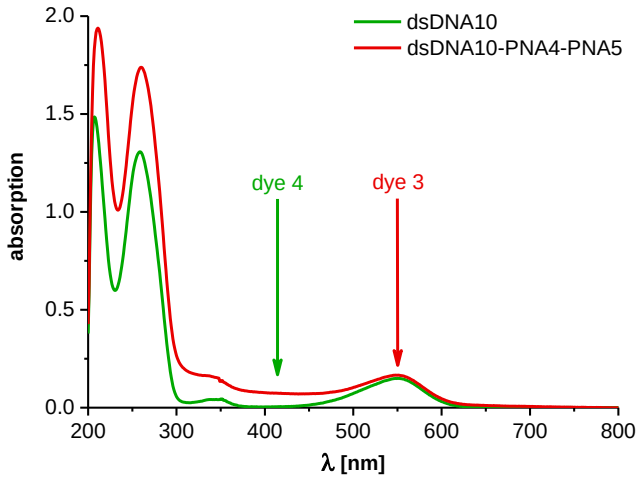
Scheme S97: Absorption spectra of strand invasion kinetic of dsDNA9-PNA4-PNA5 in absence of NaCl.



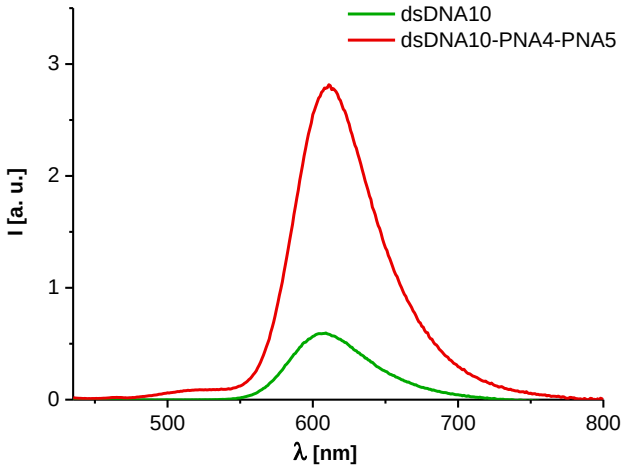
Scheme S98: Fluorescence spectra of strand invasion kinetic of dsDNA9-PNA4-PNA5 in absence of NaCl.



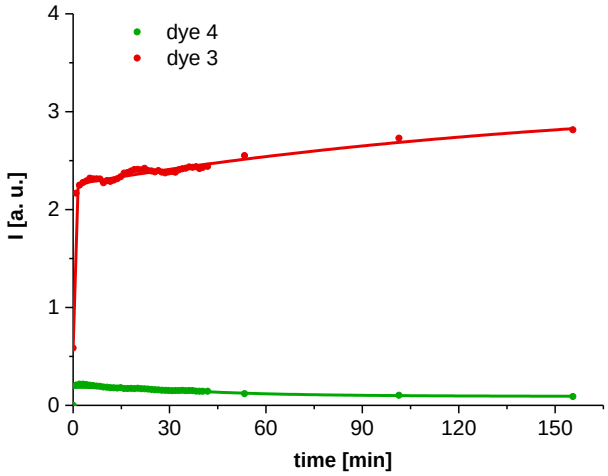
Scheme S99: Fluorescence intensity changes of dye 4 and dye 3 of strand invasion kinetic of dsDNA9-PNA4-PNA5 in absence of NaCl.



Scheme S100: Absorption spectra of strand invasion kinetic of dsDNA10-PNA4-PNA5 in presence of NaCl.

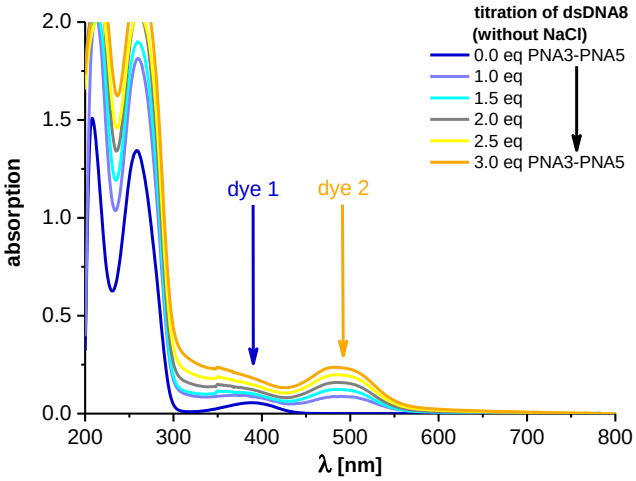


Scheme S101: Fluorescence spectra of strand invasion kinetic of dsDNA10-PNA4-PNA5 in presence of NaCl.

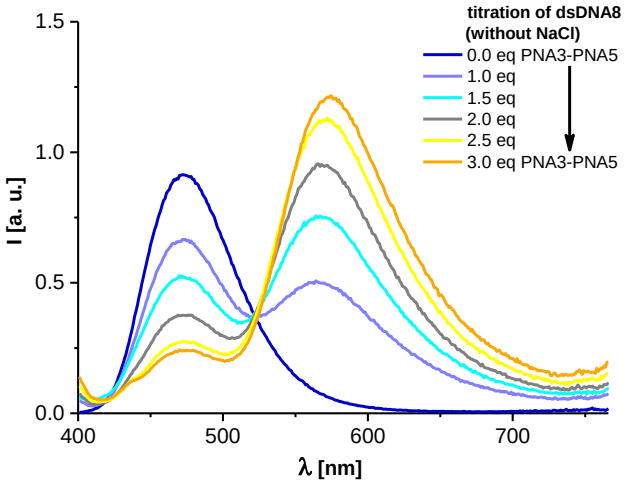


Scheme S102: Fluorescence intensity changes of dye 4 and dye 3 of strand invasion kinetic of dsDNA10-PNA4-PNA5 in presence of NaCl.

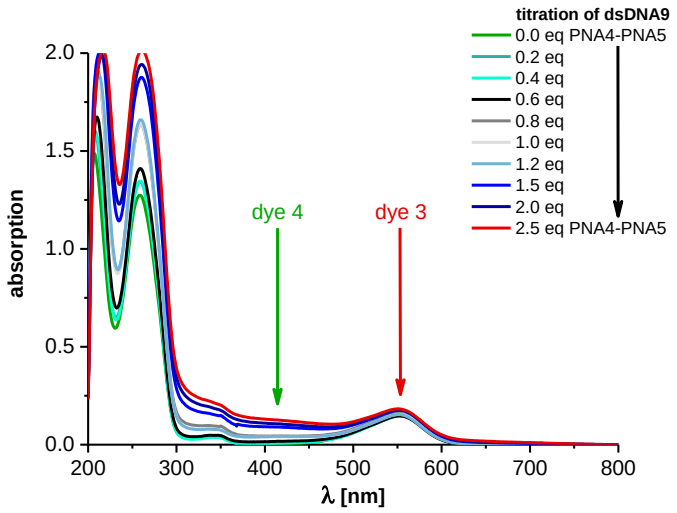
6.2.2 Spectroscopic data of titration experiments:



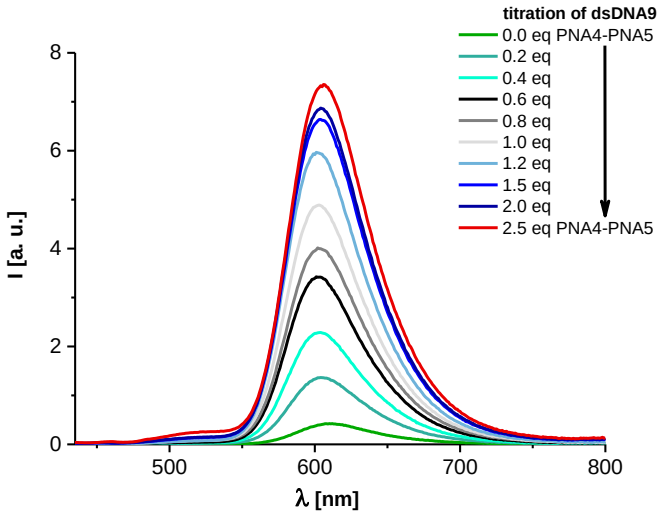
Scheme S103: Absorption spectra of strand invasion titration of dsDNA8-PNA3-PNA5 in absence of NaCl.



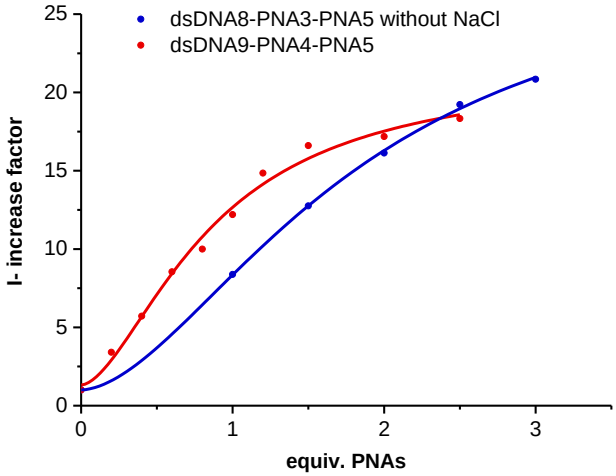
Scheme S104: Fluorescence spectra of strand invasion titration of dsDNA8-PNA3-PNA5 in absence of NaCl.



Scheme S105: Absorption spectra of strand invasion titration of dsDNA9-PNA4-PNA5 in presence of NaCl.

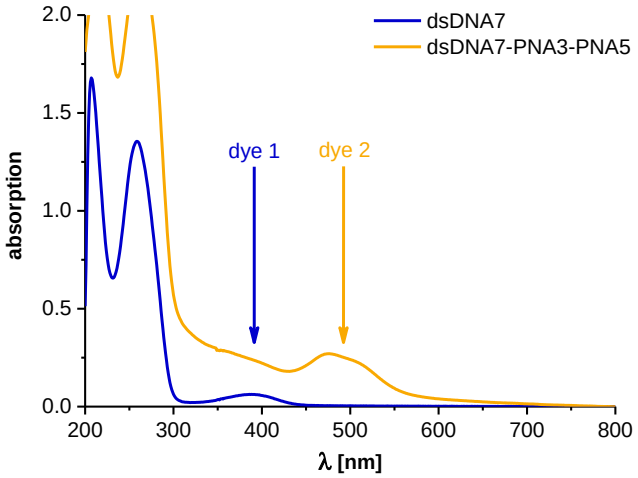


Scheme S106: Fluorescence spectra of strand invasion titration of dsDNA9-PNA4-PNA5 in presence of NaCl.

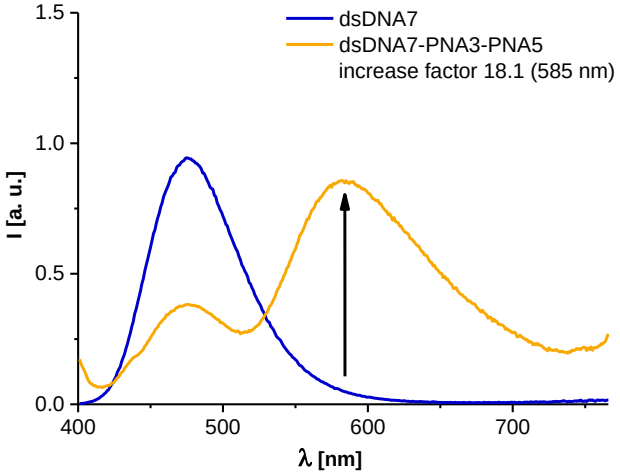


Scheme S107: Enhancement of fluorescence intensity increase factor in course of strand invasion titration of dsDNA8-PNA3-PNA5 in absence of NaCl and of dsDNA9-PNA4-PNA5 in presence of NaCl.

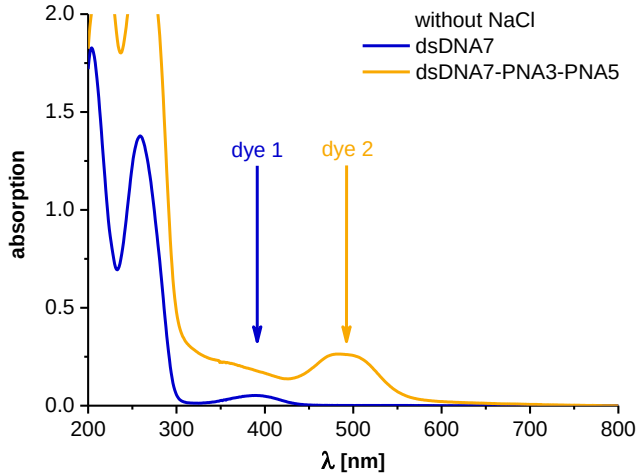
6.2.3 Spectroscopic data of final invasion measurements:



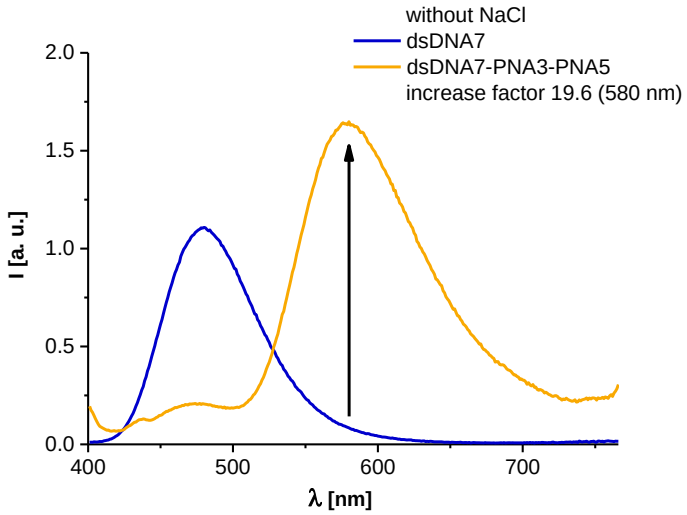
Scheme S108: Absorption spectra of final strand invasion measurement of dsDNA7-PNA3-PNA5 in presence of NaCl.



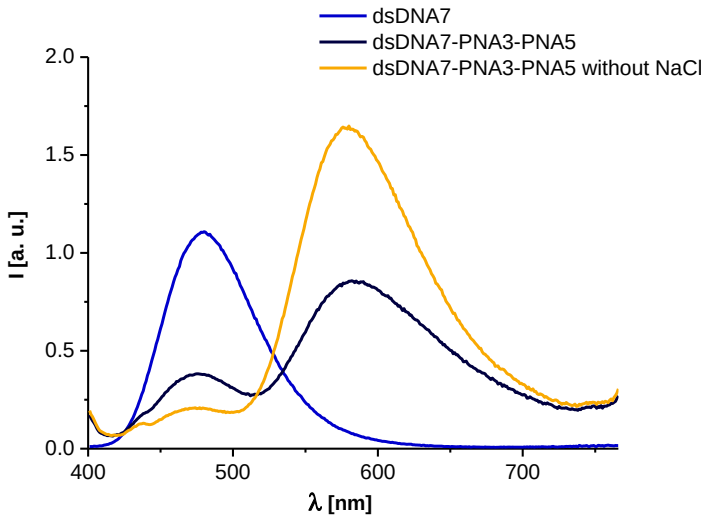
Scheme S109: Fluorescence spectra of final strand invasion measurement of dsDNA7-PNA3-PNA5 in presence of NaCl.



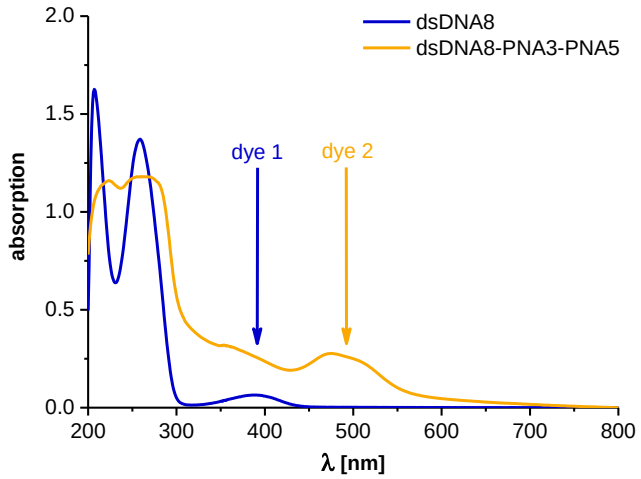
Scheme S110: Absorption spectra of final strand invasion measurement of dsDNA7-PNA3-PNA5 in absence of NaCl.



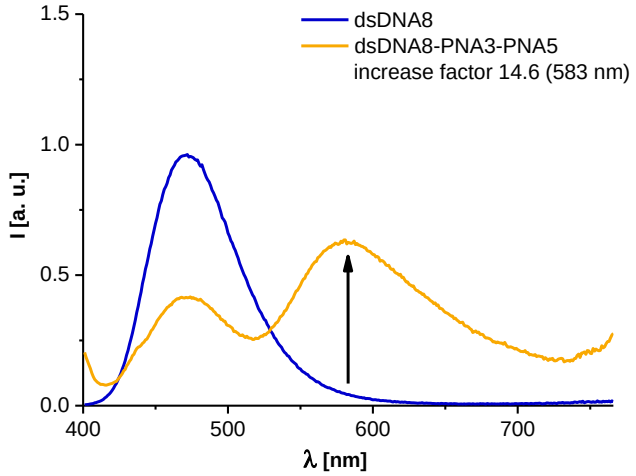
Scheme S111: Fluorescence spectra of final strand invasion measurement of dsDNA7-PNA3-PNA5 in absence of NaCl.



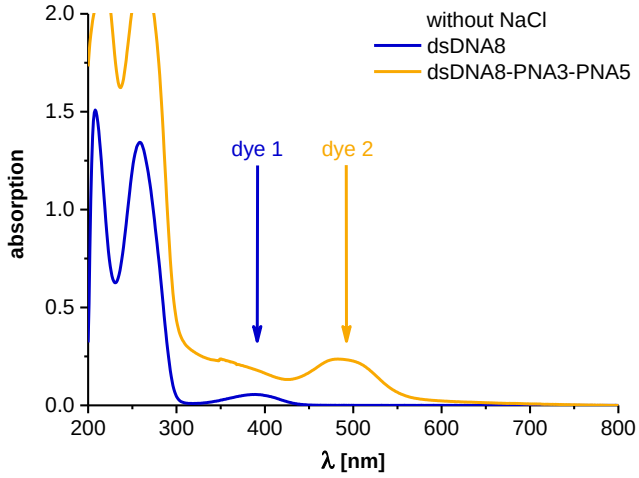
Scheme S112: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA7-PNA3-PNA5 in presence and absence of NaCl, respectively.



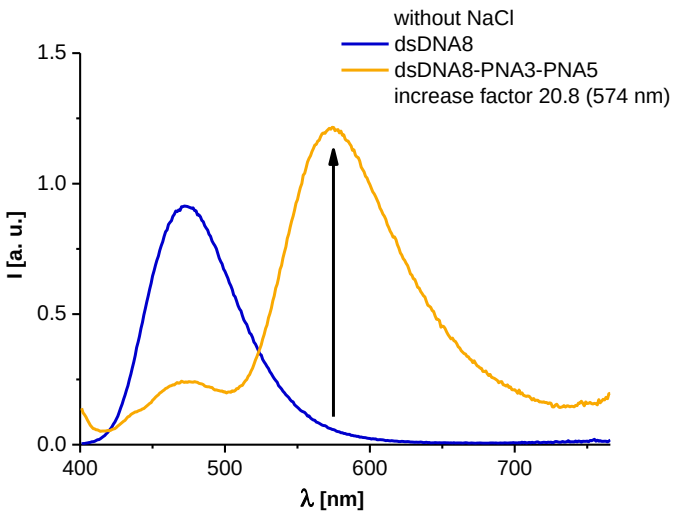
Scheme S113: Absorption spectra of final strand invasion measurement of dsDNA8-PNA3-PNA5 in presence of NaCl.



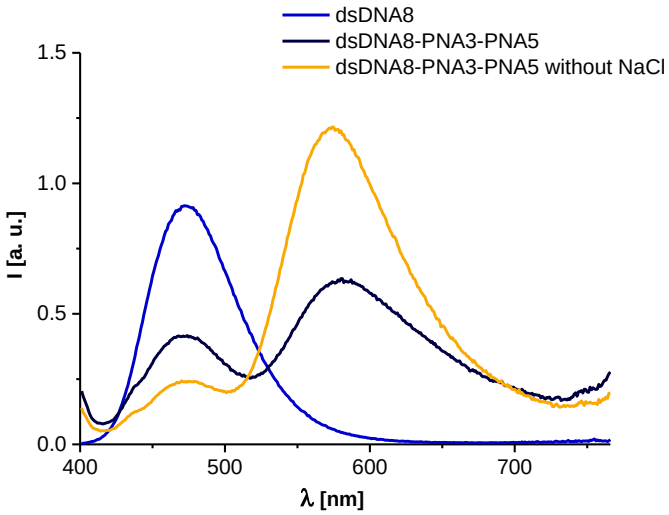
Scheme S114: Fluorescence spectra of final strand invasion measurement of dsDNA8-PNA3-PNA5 in presence of NaCl.



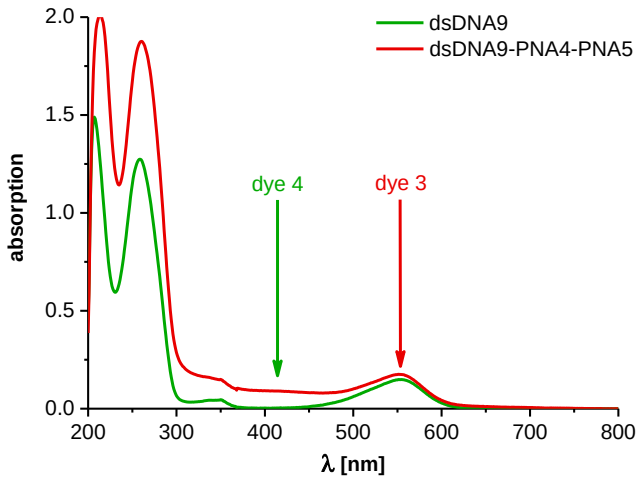
Scheme S115: Absorption spectra of final strand invasion measurement of dsDNA8-PNA3-PNA5 in absence of NaCl.



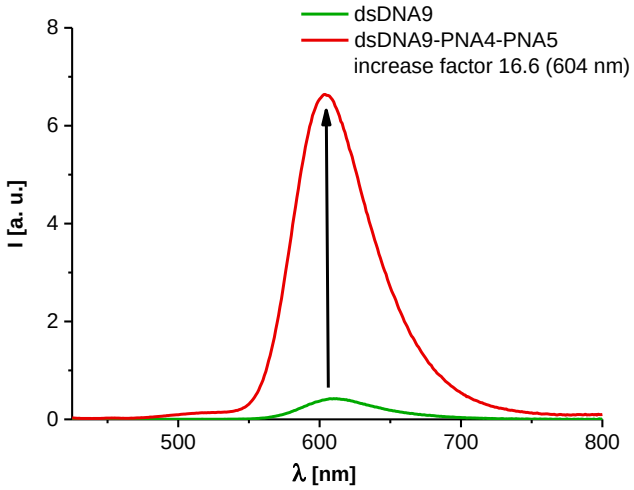
Scheme S116: Fluorescence spectra of final strand invasion measurement of dsDNA8-PNA3-PNA5 in absence of NaCl.



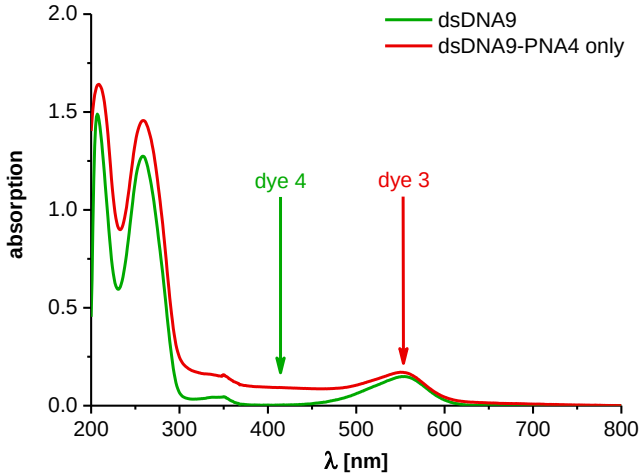
Scheme S117: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA8-PNA3-PNA5 in presence and absence of NaCl, respectively.



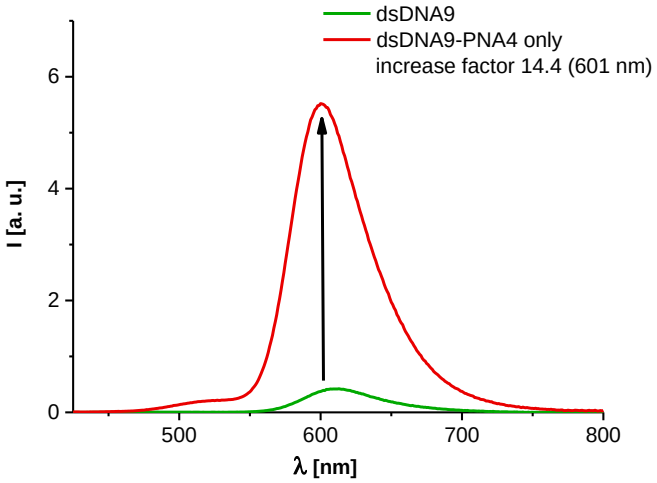
Scheme S118: Absorption spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 in presence of NaCl.



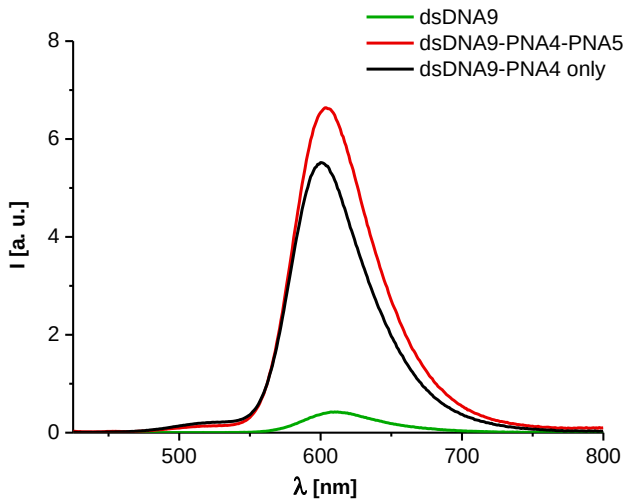
Scheme S119: Fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 in presence of NaCl.



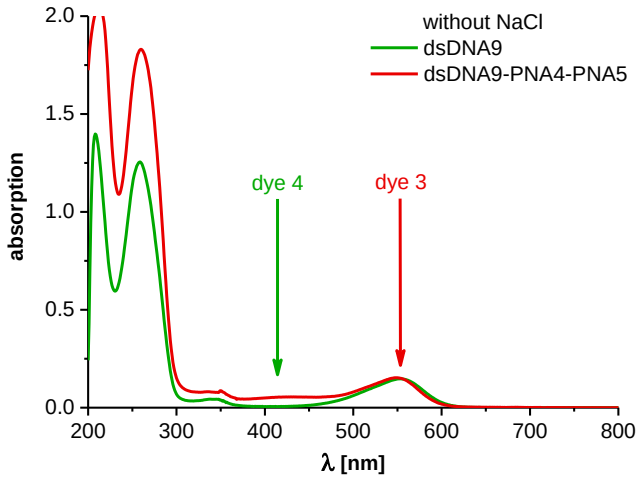
Scheme S120: Absorption spectra of final strand invasion measurement of dsDNA9-PNA4 in presence of NaCl.



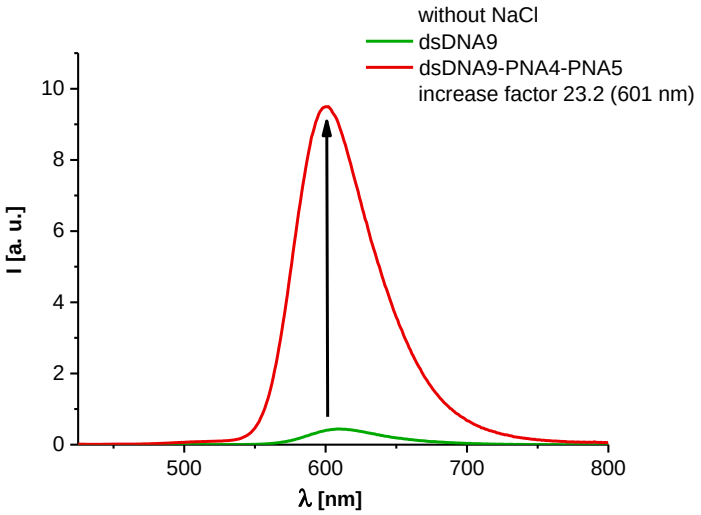
Scheme S121: Fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4 in presence of NaCl.



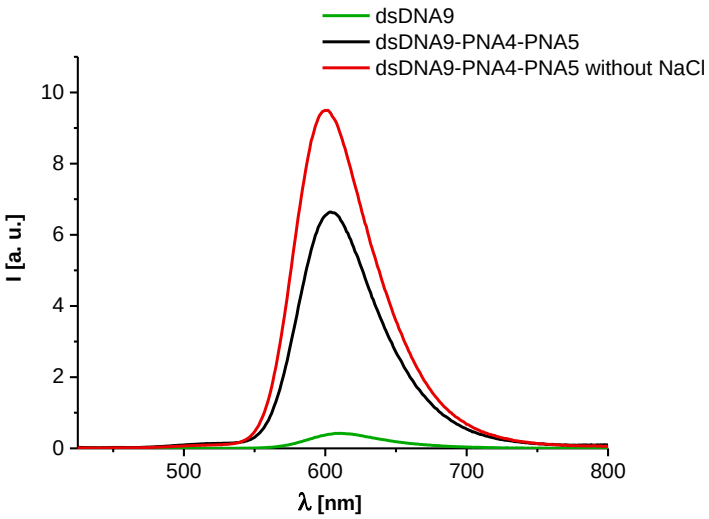
Scheme S122: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 and dsDNA9-PNA4 in presence of NaCl, respectively.



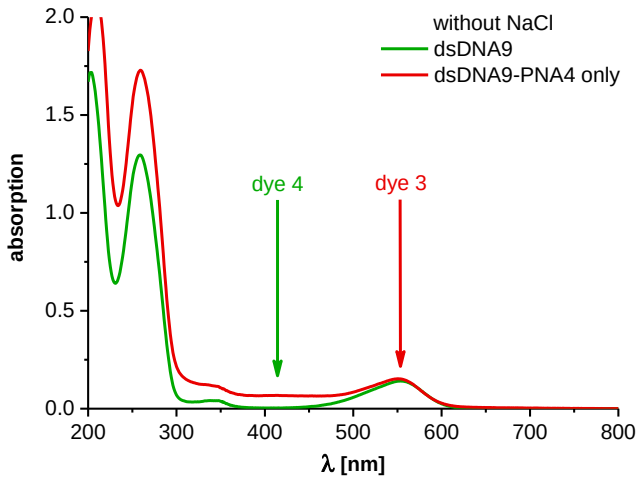
Scheme S123: Absorption spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 in absence of NaCl.



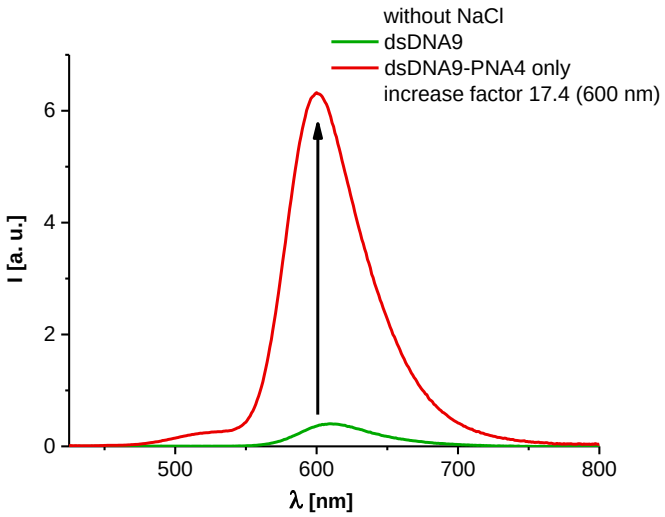
Scheme S124: Fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 in absence of NaCl.



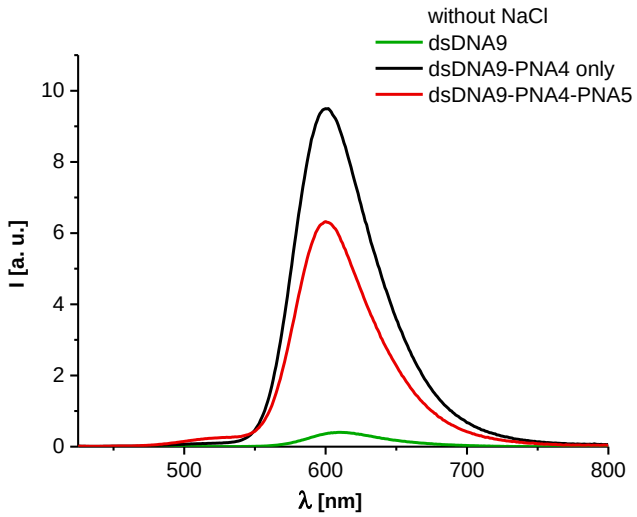
Scheme S125: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 in presence and absence of NaCl, respectively.



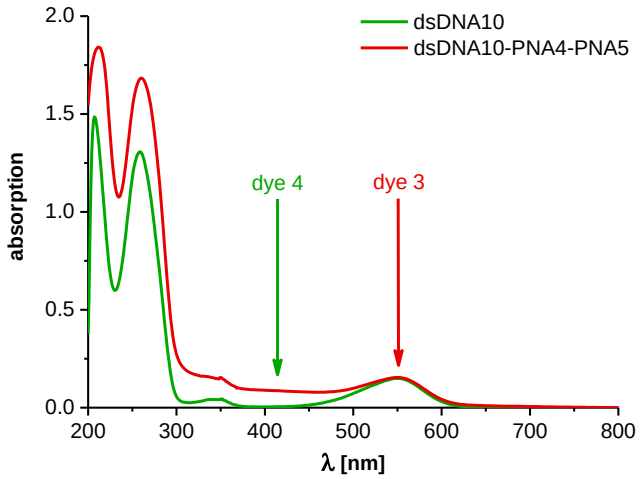
Scheme S126: Absorption spectra of final strand invasion measurement of dsDNA9-PNA4 in absence of NaCl.



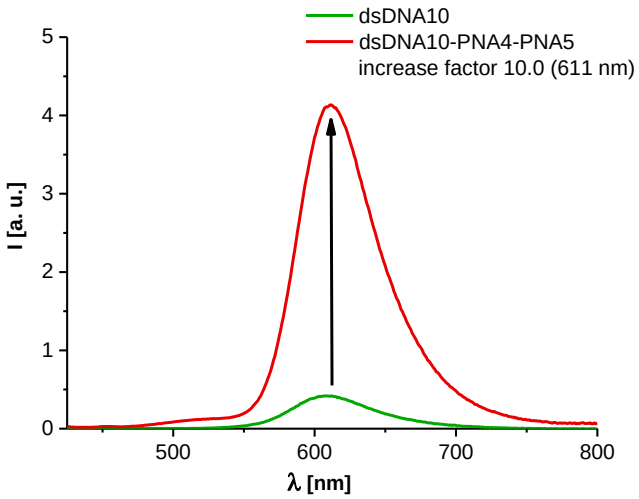
Scheme S127: Fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4 in absence of NaCl.



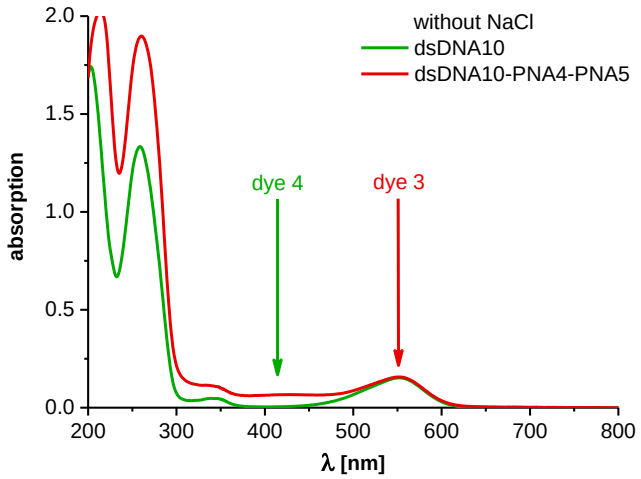
Scheme S128: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4-PNA5 and dsDNA9-PNA4 in absence of NaCl, respectively.



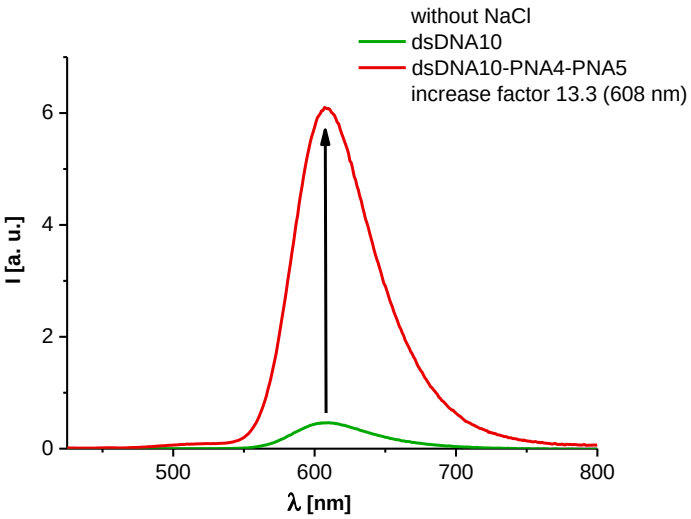
Scheme S129: Absorption spectra of final strand invasion measurement of dsDNA10-PNA4-PNA5 in presence of NaCl.



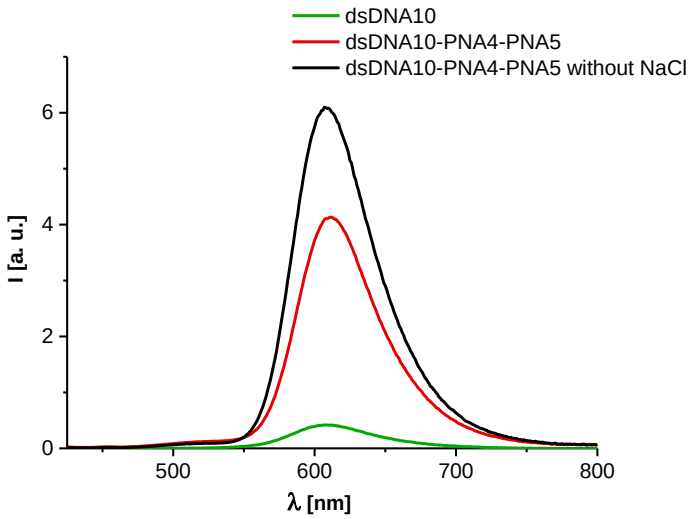
Scheme S130: Fluorescence spectra of final strand invasion measurement of dsDNA10-PNA4-PNA5 in presence of NaCl.



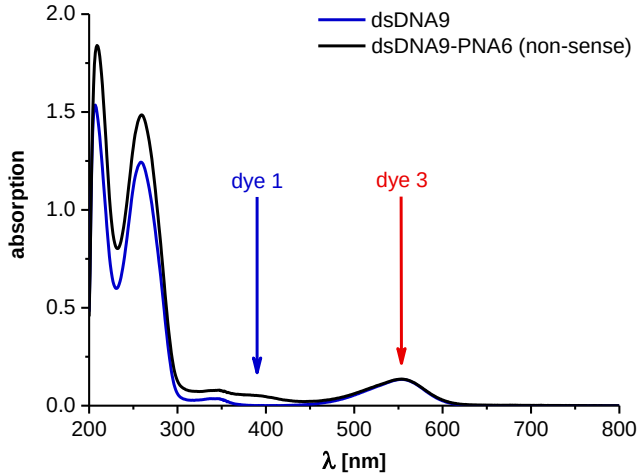
Scheme S131: Absorption spectra of final strand invasion measurement of dsDNA10-PNA4-PNA5 in absence of NaCl.



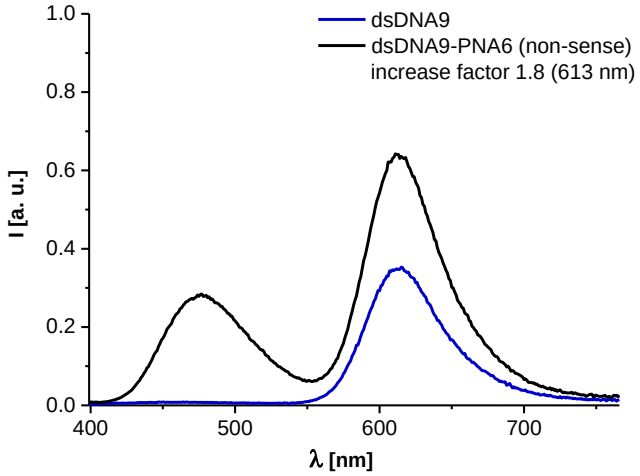
Scheme S132: Fluorescence spectra of final strand invasion measurement of dsDNA10-PNA4-PNA5 in absence of NaCl.



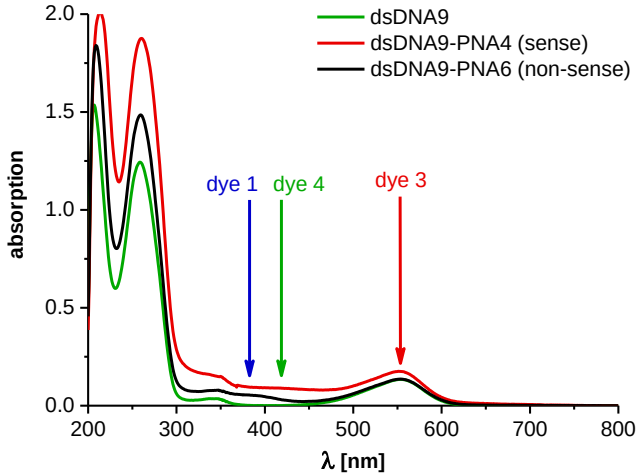
Scheme S133: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA10-PNA4-PNA5 in presence and absence of NaCl, respectively.



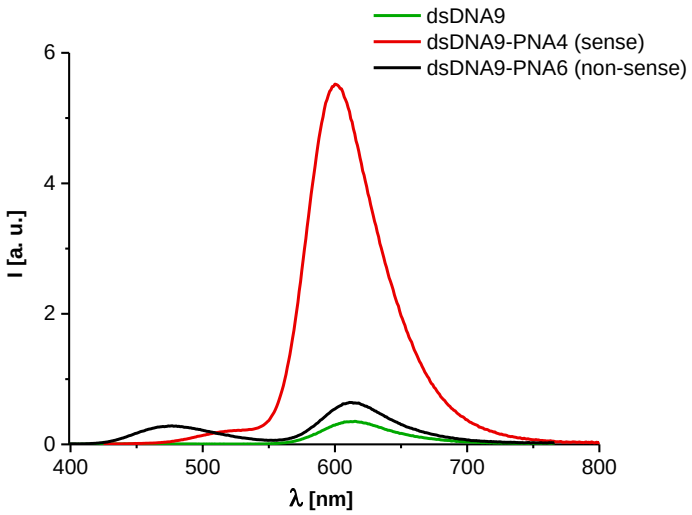
Scheme S134: Absorption spectra of final strand invasion measurement of dsDNA9 with non-sense PNA6 in presence of NaCl.



Scheme S135: Fluorescence spectra of final strand invasion measurement of dsDNA9 with non-sense PNA6 in presence of NaCl.

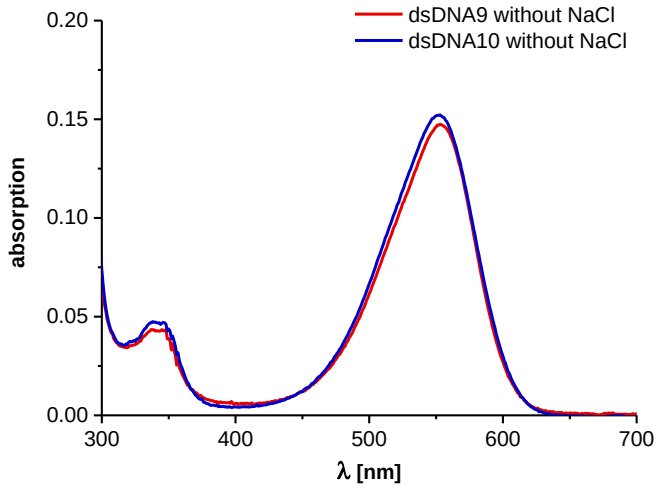


Scheme S136: Comparison of absorption spectra of final strand invasion measurement of dsDNA9-PNA4 (sense) and dsDNA9 with non-sense PNA6 in presence of NaCl, respectively.

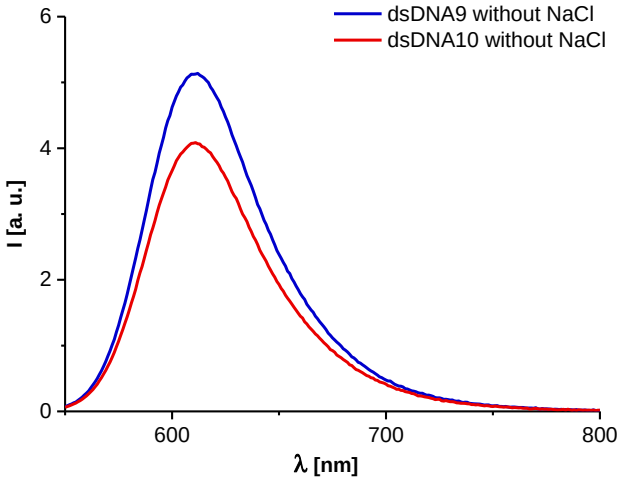


Scheme S137: Comparison of fluorescence spectra of final strand invasion measurement of dsDNA9-PNA4 (sense) and dsDNA9 with non-sense PNA6 in presence of NaCl, respectively.

6.2.4 Comparison of dye 3 attachment at adenosine and uridine:



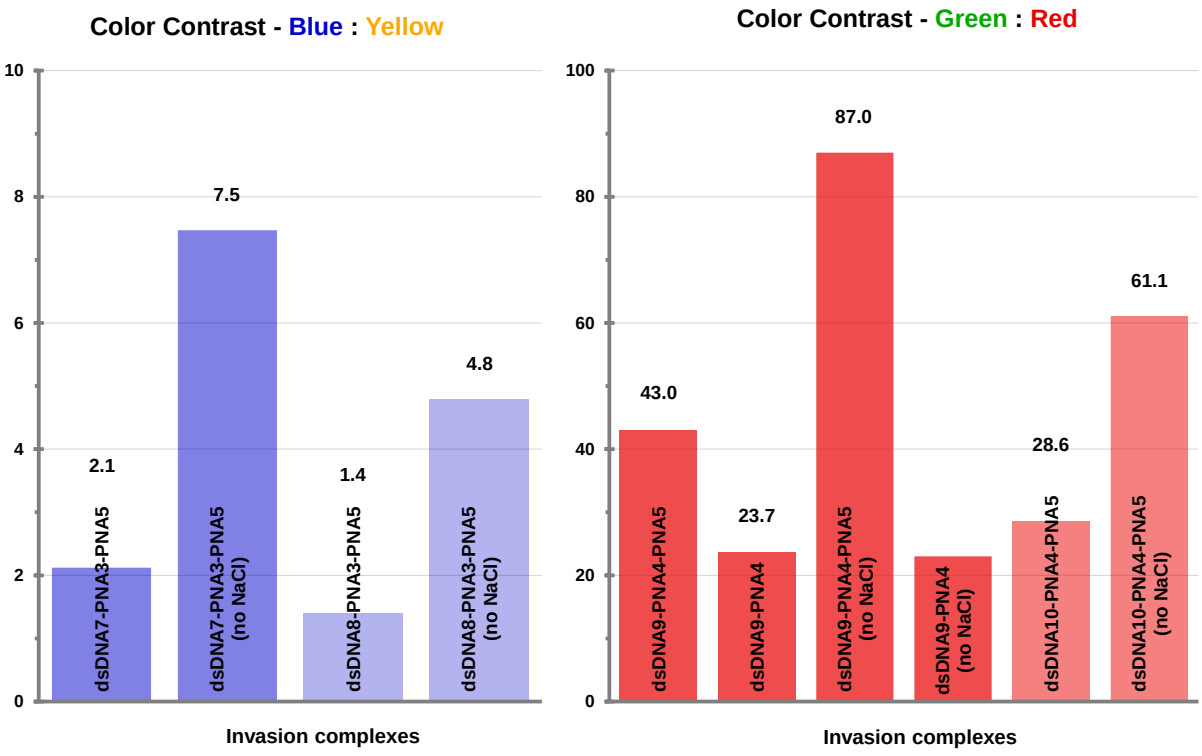
Scheme S138: Comparison of absorption spectra of dsDNA9 (dye 3 at 2´of adenosine) and dsDNA10 (dye 3 at 2´of uridine) in absence of NaCl, respectively.



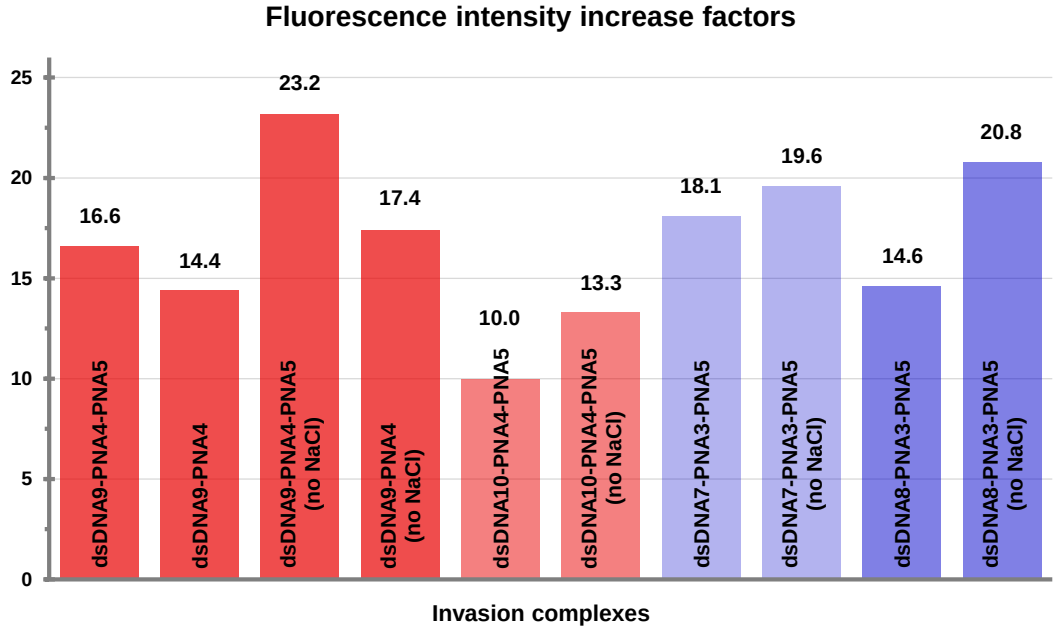
Scheme S139: Comparison of fluorescence spectra of dsDNA9 (dye 3 at 2´of adenosine) and dsDNA10 (dye 3 at 2´of uridine) in absence of NaCl, respectively.

6.3 Color contrasts and fluorescence intensity increase factors:

Fluorescence intensity increase factors were calculated by determined fluorescence intensity enhancement from each value of sample solution before invasion to final fluorescence intensity of sample solution after invasion, respectively.



Scheme S140: Fluorescence color contrasts of strand invasion. Color contrasts blue:yellow (left) and green:red (right).



Scheme S141: Fluorescence intensity increase factors of strand invasion.

6.4 ET-efficiencies, fluorescence lifetimes and Invasion efficiencies:

Fluorescence lifetimes were determined of invasion complexes with 2.5 µM dsDNA7 – dsDNA10 in NaP_i buffer solution (10 mM, pH = 7), with 3.0 equivalents of PNA3 and PNA5 (dsDNA7, dsDNA8) or 1.5 equivalents of PNA4 and / or PNA5 (dsDNA9, dsDNA10) in absence and presence of 250 mM NaCl, respectively. Lifetimes of donor dye 1 were recorded of 2.5 µM dsDNA7 and dsDNA8 in NaP_i buffer solution (10 mM, pH = 7) in absence and presence of 250 mM NaCl, respectively. Lifetimes of donor dye 4 were recorded of 2.5 µM PNA4-DNA21 in NaP_i buffer solution (10 mM, pH = 7) in absence and presence of 250 mM NaCl, respectively.

In order to calculate invasion efficiencies fluorescence lifetimes of additional compare solutions were recorded. These compare solutions contained 2.5 µM DNA17 or DNA18 3.0 equivalents PNA3; or DNA19 or DNA20 and 1.5 equivalents PNA4 in absence or presence of NaCl, respectively.

Fluorescence lifetimes were recorded with Horiba Scientific FluoroMax-4 spectrofluorometer using a time-correlated single photon counting (TCSPC) technique with excitation sources NanoLed at 370 nm for dye 1 or 455 nm for dye 4 (Horiba, impulse repetition rate of 1 MHz, time calibration = 2.74E-11 sec/ch) and emission intensities were detected at 475 nm for dye 1 or 528 nm for dye 4. Lifetimes were calculated with DAS6 v 6.8 decay analysis software (Horiba), mono-exponential fit for donor lifetimes in absence of acceptor dye and bi- exponential fit for donor lifetimes in presence of acceptor dye.

Energy transfer efficiencies were calculated by following equation:

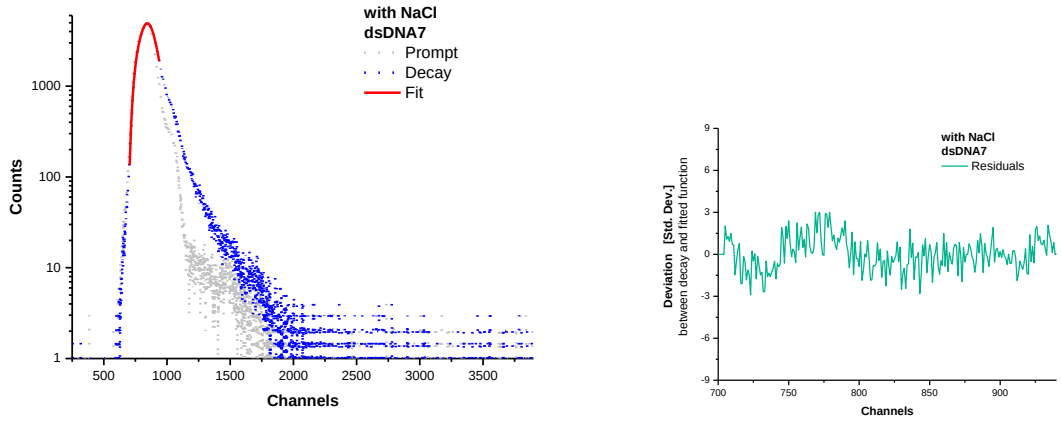
$$\boldsymbol{E_{transfer} = 1 - \frac{\tau_{DA}}{\tau_D}}$$

With: $\boldsymbol{\tau_{DA}}$ = lifetime of donor dye 1 (or dye 4) in presence of acceptor dye
$\boldsymbol{\tau_D}$ = lifetime of donor dye 1 (or dye 4) in absence of acceptor dye

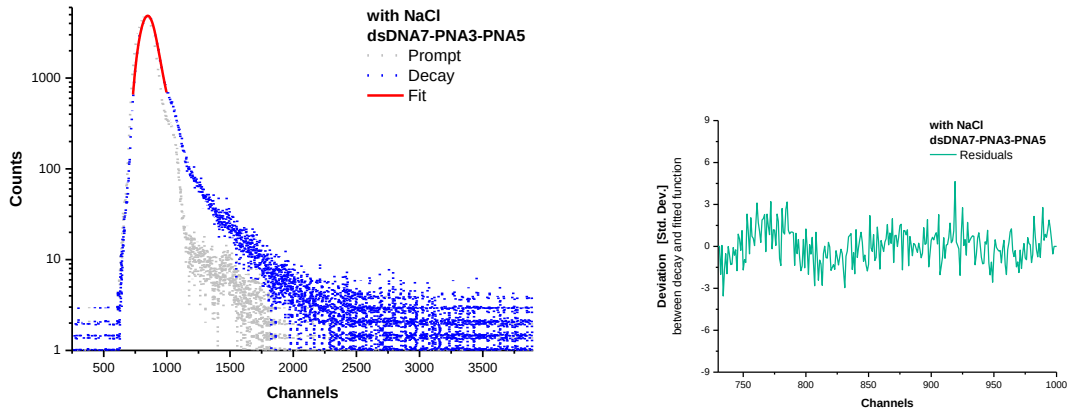
Invasion efficiencies were determined as the quotient of the energy transfer efficiencies of each invasion complex and the corresponding compare solution:

$$\boldsymbol{E_{invasion} = \frac{E_{transfer,\,invasion\,complex}}{E_{transfer,\,compare\,solution}}}$$

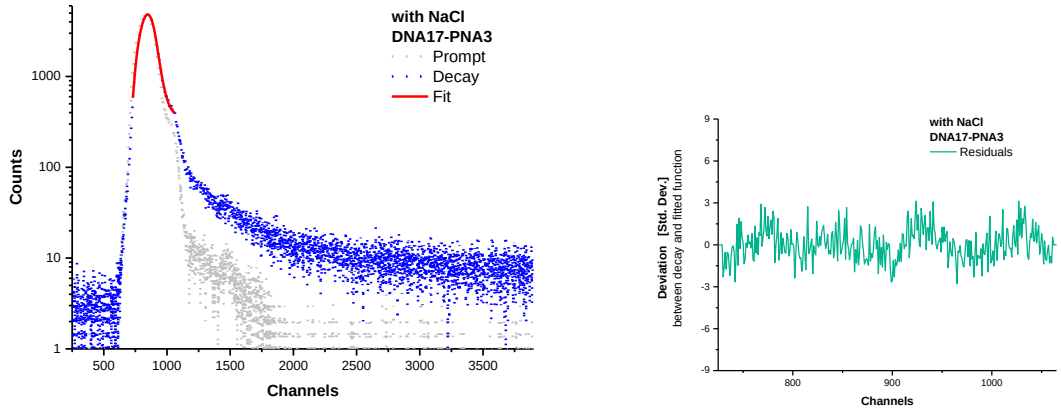
τ_{dye1}-Decay graphs of strand invasion complexes with PNA3/PNA5:



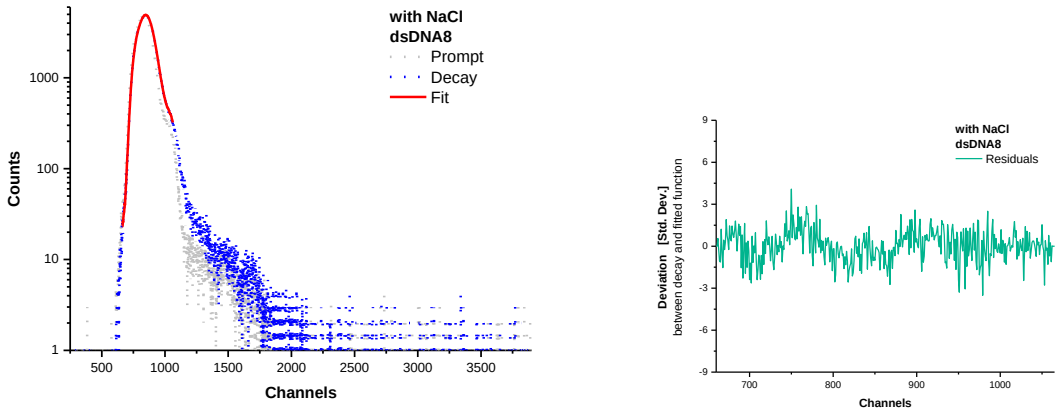
Scheme S142: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasions dsDNA7 in presence of NaCl.



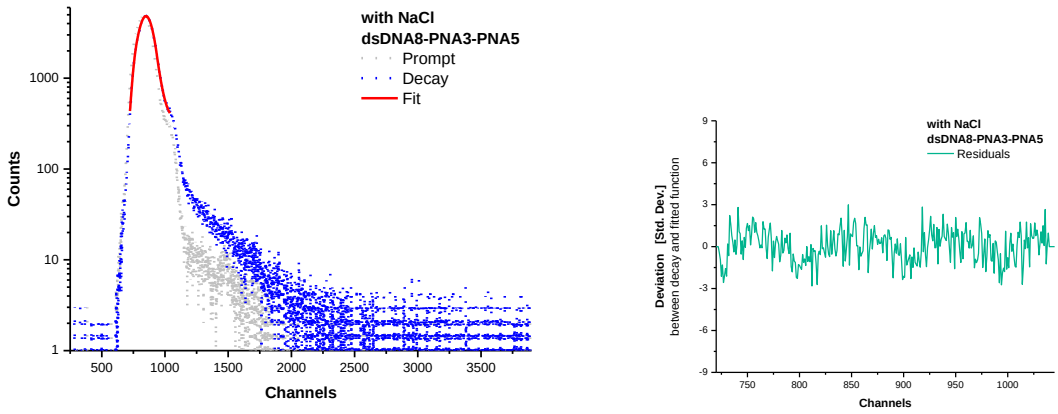
Scheme S143: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion complex dsDNA7 with 3.0 equivalents PNA3/PNA5 in presence of NaCl.



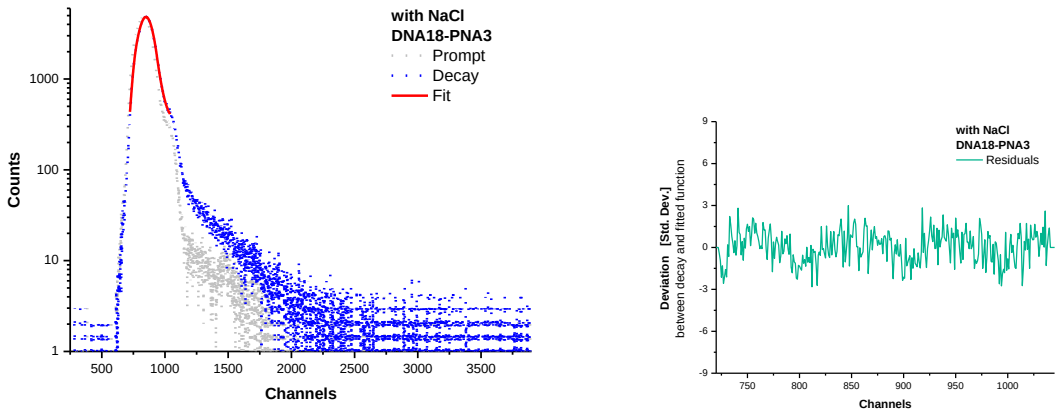
Scheme S144: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion compare solution with DNA17 and 3.0 equivalents PNA3 in presence of NaCl.



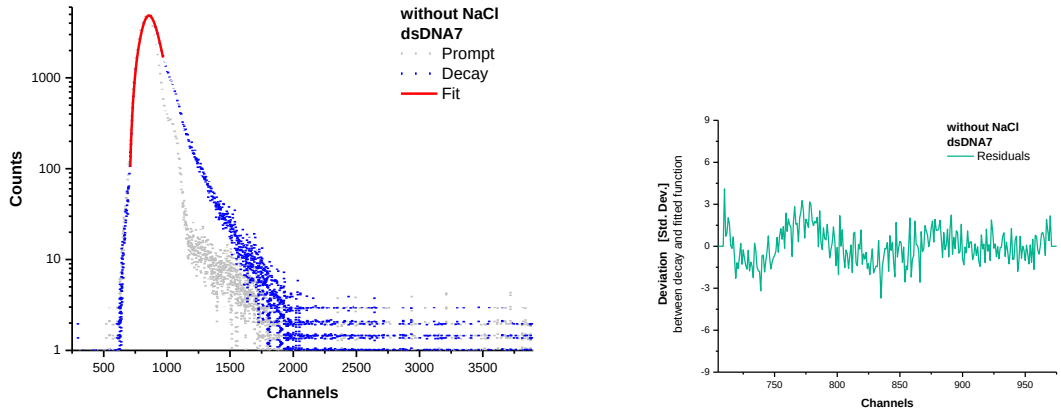
Scheme S145: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasions dsDNA8 in presence of NaCl.



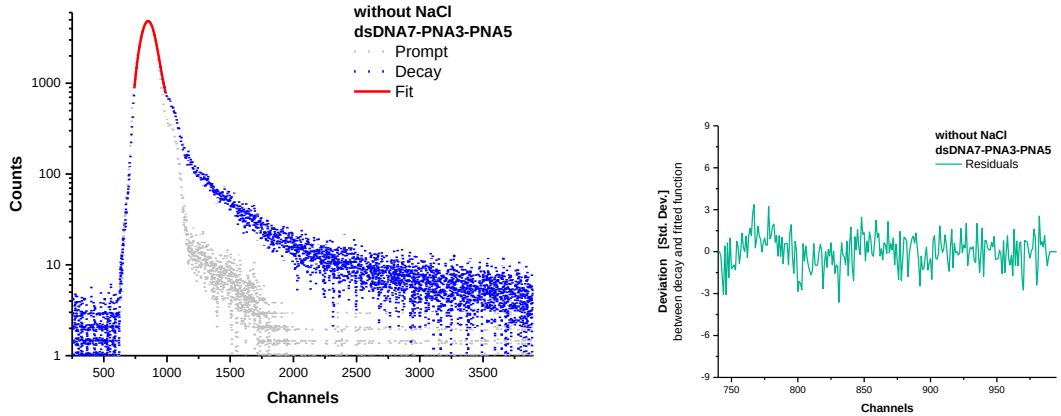
Scheme S146: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion complex dsDNA8 with 3.0 equivalents PNA3/PNA5 in presence of NaCl.



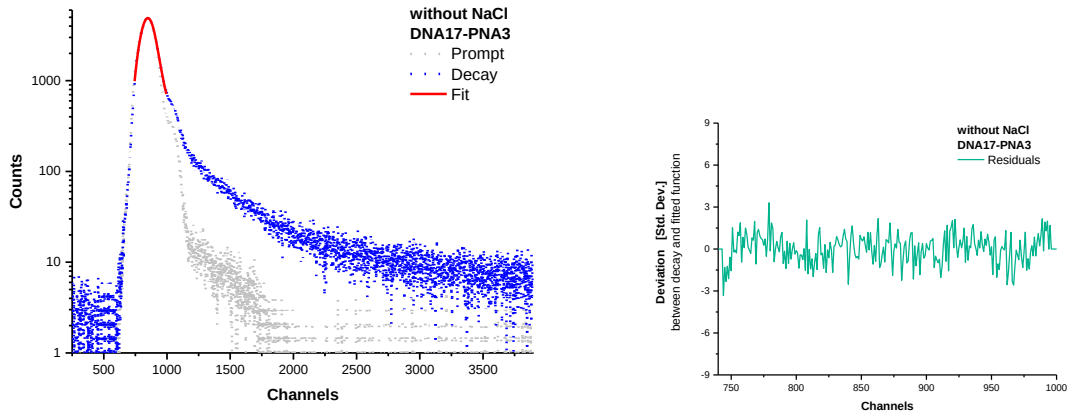
Scheme S147: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion compare solution with DNA18 and 3.0 equivalents PNA3 in presence of NaCl.



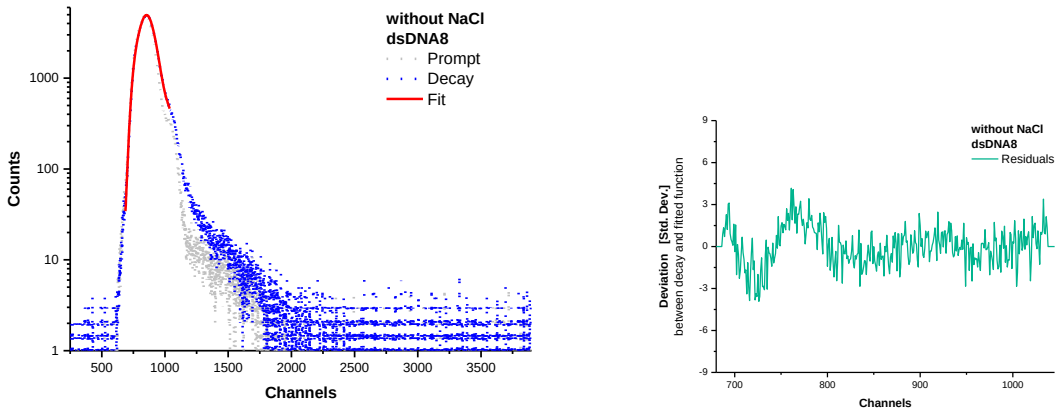
Scheme S148: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasions dsDNA7 in absence of NaCl.



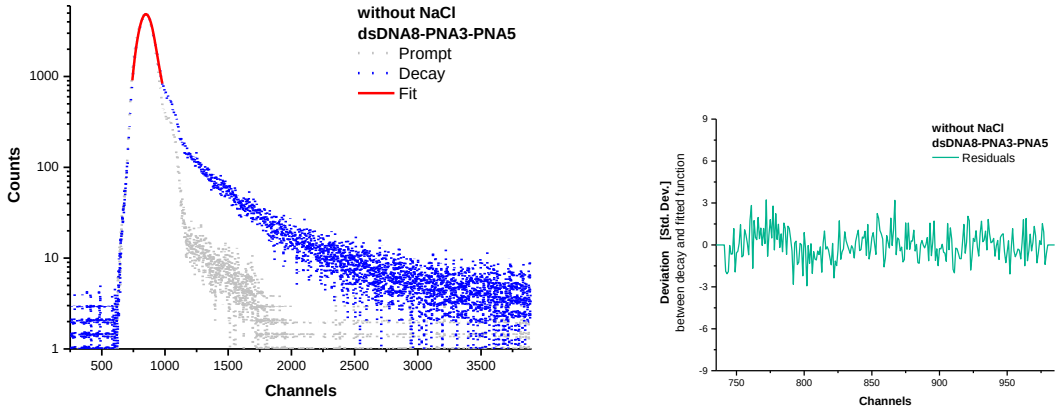
Scheme S149: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion complex dsDNA7 with 3.0 equivalents PNA3/PNA5 in absence of NaCl.



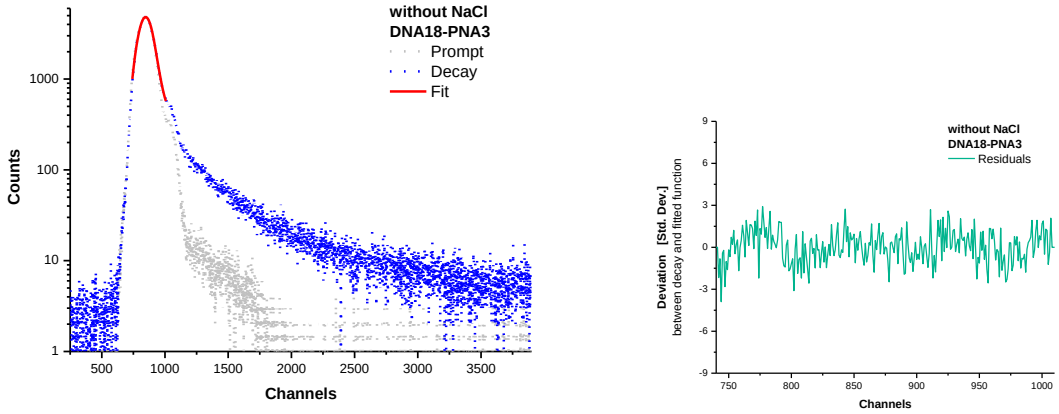
Scheme S150: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion compare solution with DNA17 and 3.0 equivalents PNA3 in absence of NaCl.



Scheme S151: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasions dsDNA8 in absence of NaCl.



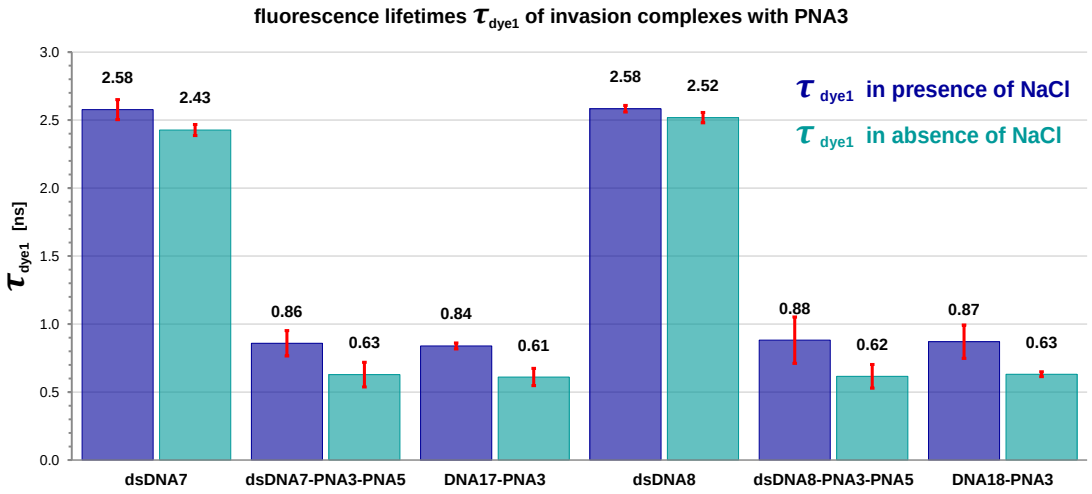
Scheme S152: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion complex dsDNA8 with 3.0 equivalents PNA3/PNA5 in absence of NaCl.



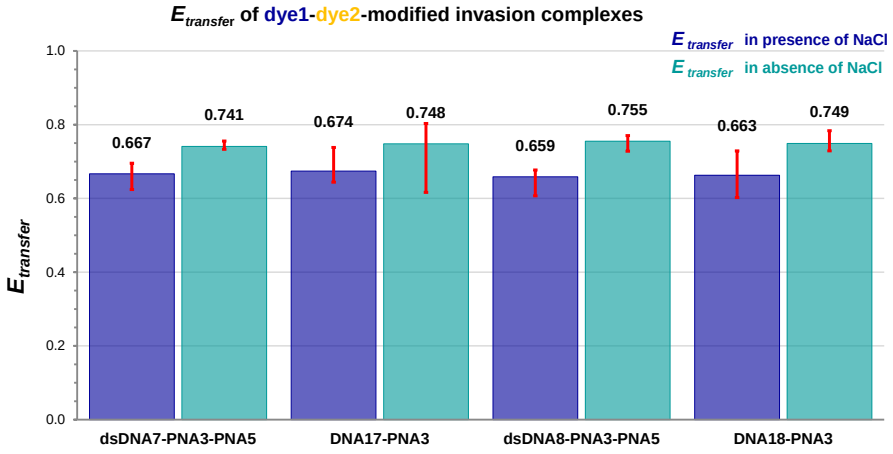
Scheme S153: Fluorescence lifetime decay of donor dye 1 (left) and residuals (right) of strand invasion compare solution with DNA18 and 3.0 equivalents PNA3 in absence of NaCl.

Invasion Efficiency into dsDNA by PNA3, PNA5 in presence and absence of NaCl:											Δ = Standard Deviation
Conditions	Duplex / Complex	τ_{dye1} [ns]	$\Delta\tau_{dye1}$ [ns]	$\Delta\tau_{dye1}$ [%]	$E_{transfer}$	$\Delta E_{transfer}$ [%]	$\Delta E_{transfer}$	$E_{invasion}$	$\Delta E_{invasion}$ [%]	$\Delta E_{invasion}$	
with NaCl	dsDNA7	2.577	0.074	2.87	-	-	-	-	-	-	
	dsDNA7-PNA3-PNA5	0.859	0.093	10.89	0.667	13.76	0.092	0.989	19.25	0.129	
	DNA17-PNA3	0.840	0.022	2.62	0.674	5.49	0.037	-	-	-	
	dsDNA8	2.584	0.024	0.93	-	-	-	-	-	-	
	dsDNA8-PNA3-PNA5	0.882	0.170	19.33	0.659	22.20	0.146	0.993	39.21	0.259	
	DNA18-PNA3	0.871	0.123	14.13	0.663	17.01	0.113	-	-	-	
without NaCl	dsDNA7	2.427	0.041	1.69	-	-	-	-	-	-	
	dsDNA7-PNA3-PNA5	0.628	0.091	14.44	0.741	16.12	0.119	0.991	28.20	0.210	
	DNA17-PNA3	0.611	0.063	10.39	0.748	12.08	0.090	-	-	-	
	dsDNA8	2.518	0.038	1.50	-	-	-	-	-	-	
	dsDNA8-PNA3-PNA5	0.616	0.088	14.21	0.755	15.90	0.120	1.008	20.46	0.154	
	DNA18-PNA3	0.631	0.018	2.87	0.749	4.56	0.034	-	-	-	

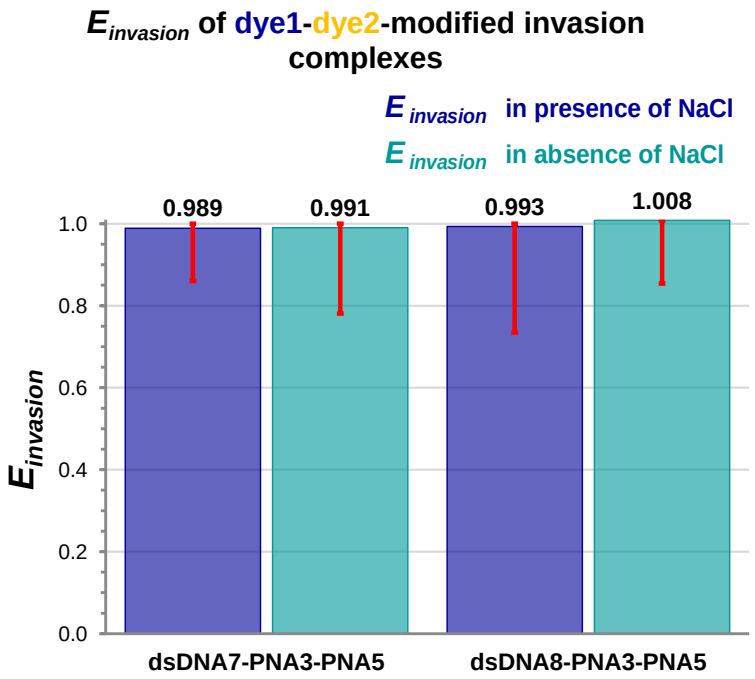
Table S3: Fluorescence lifetimes, energy transfer efficiencies and invasion efficiencies of strand invasion complexes with PNA3/PNA5.



Scheme 154: Fluorescence lifetimes of strand invasion complexes with PNA3/PNA5.

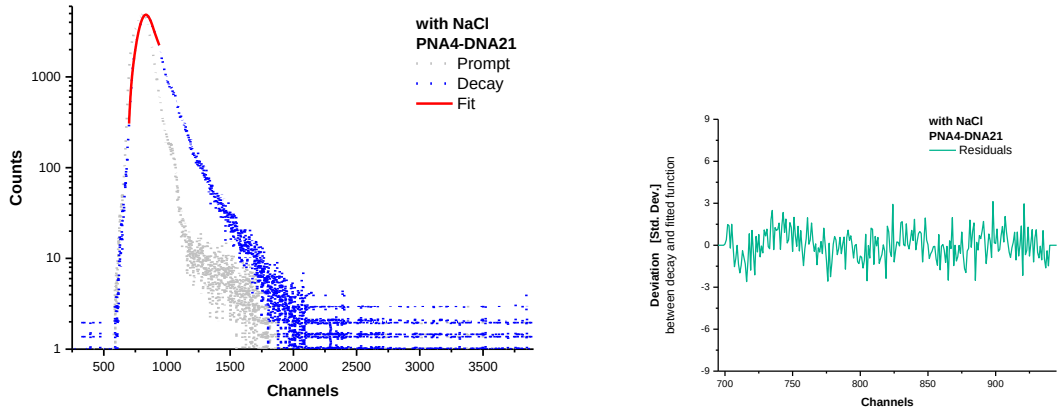


Scheme 155: Energy transfer efficiencies of strand invasion complexes with PNA3/PNA5.

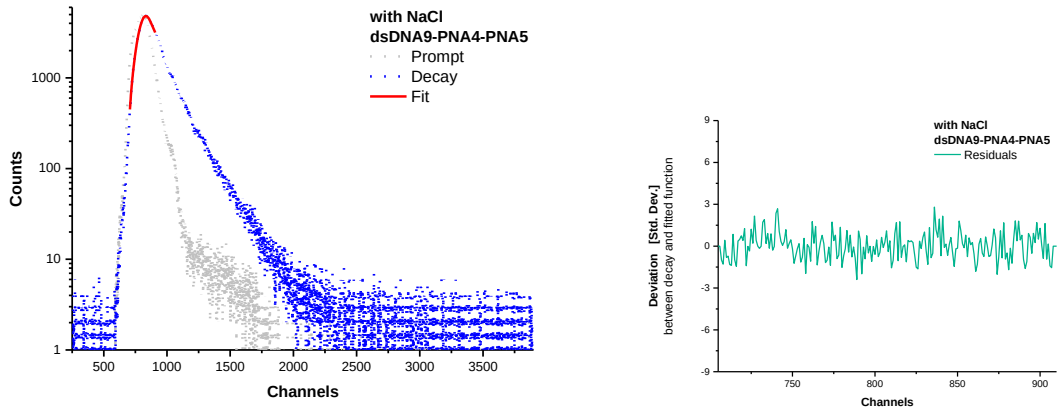


Scheme 156: Invasion efficiencies of strand invasion complexes with PNA3/PNA5.

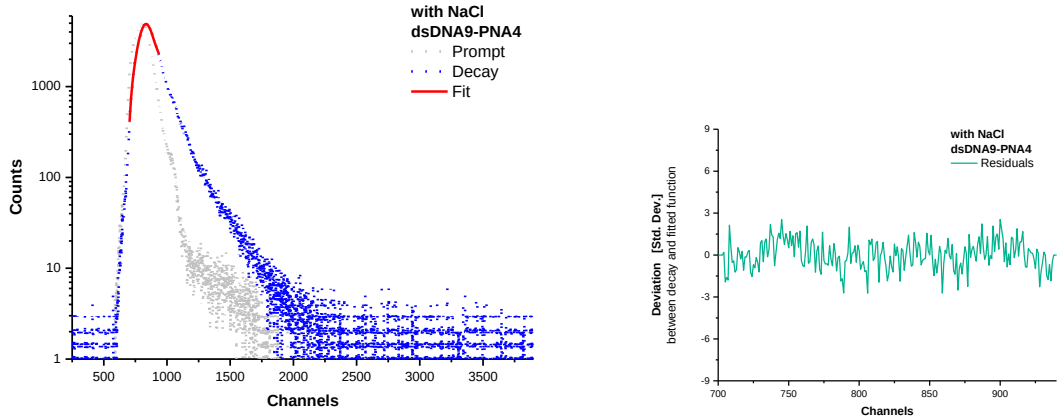
τ_{dye4}-Decay graphs of strand invasion complexes with PNA4/PNA5:



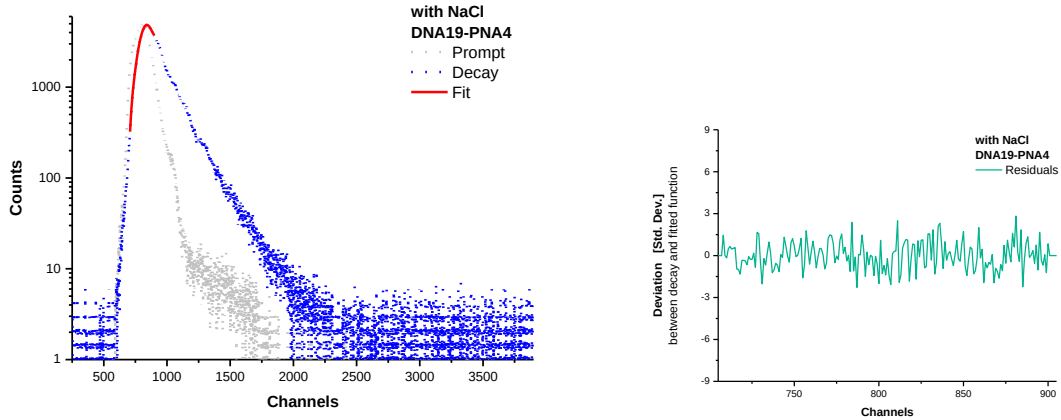
Scheme S157: Fluorescence lifetime decay of donor dye 14 (left) and residuals (right) of strand invasion PNA4-DNA21 in presence of NaCl.



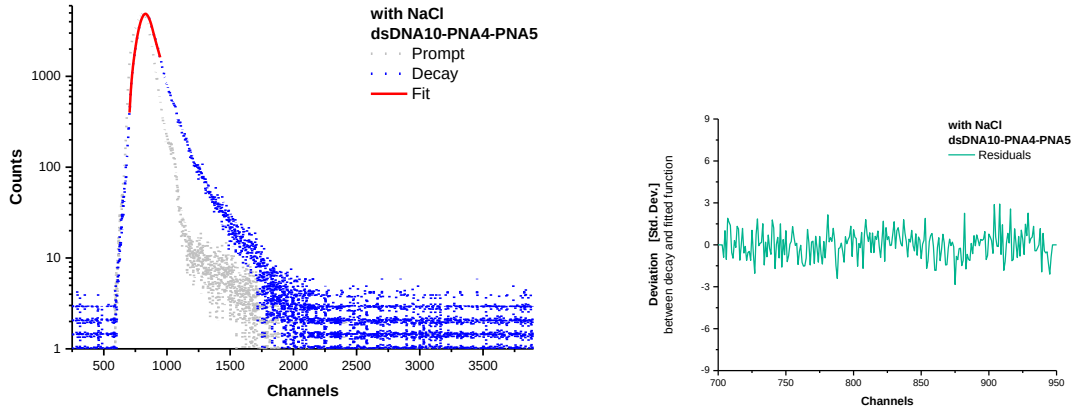
Scheme S158: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion complex dsDNA9 with 1.5 equivalents PNA4/PNA5 in presence of NaCl.



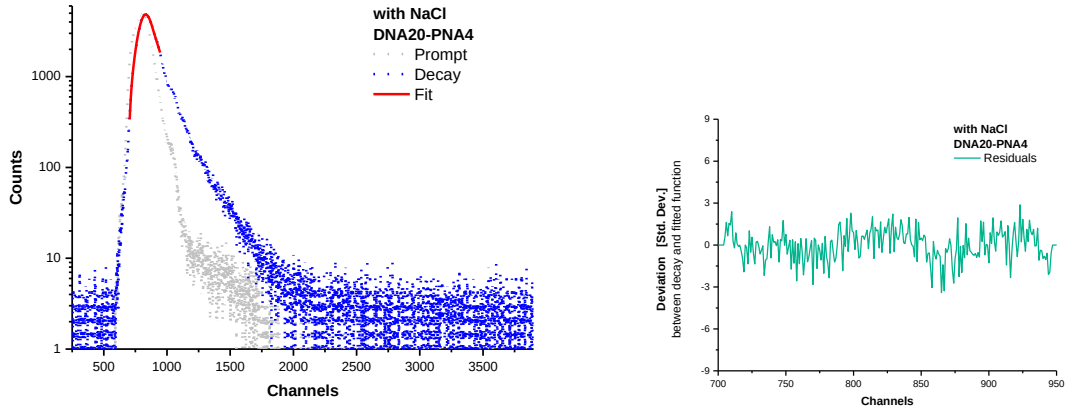
Scheme S159: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion complex dsDNA9 with 1.5 equivalents PNA4 in presence of NaCl.



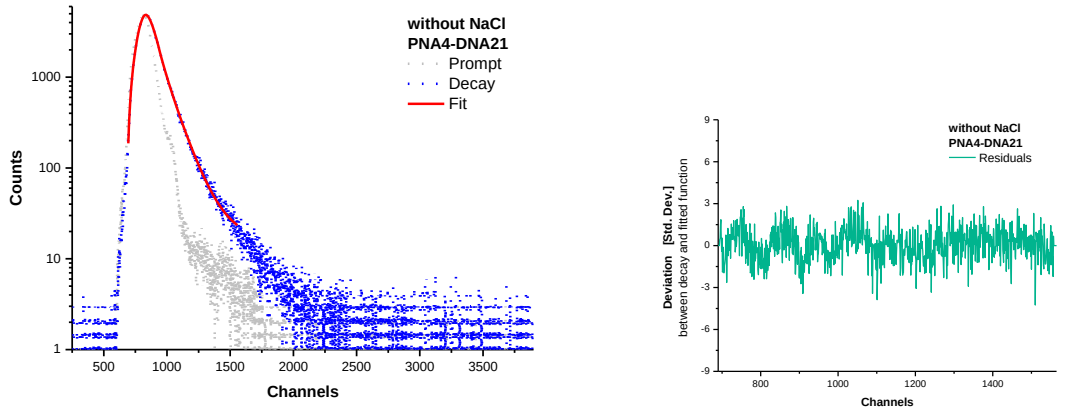
Scheme S160: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion compare solution with DNA19 and 1.5 equivalents PNA4 in presence of NaCl.



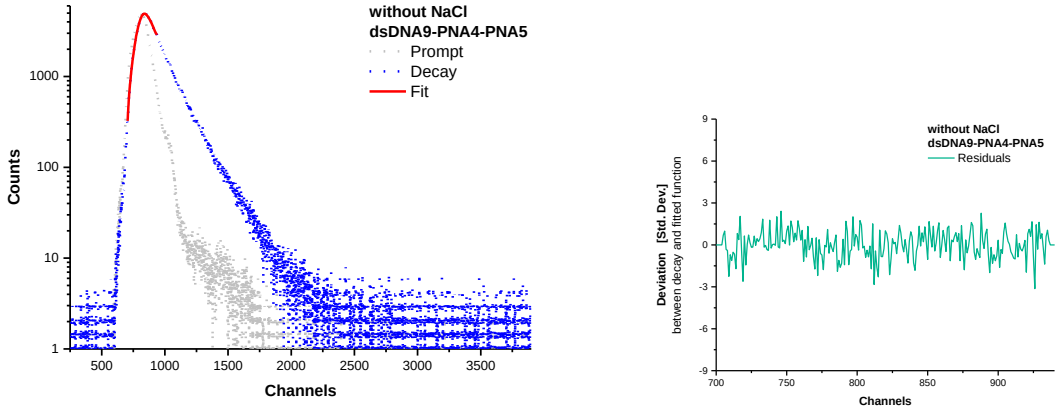
Scheme S161: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion complex dsDNA10 with 1.5 equivalents PNA4/PNA5 in presence of NaCl.



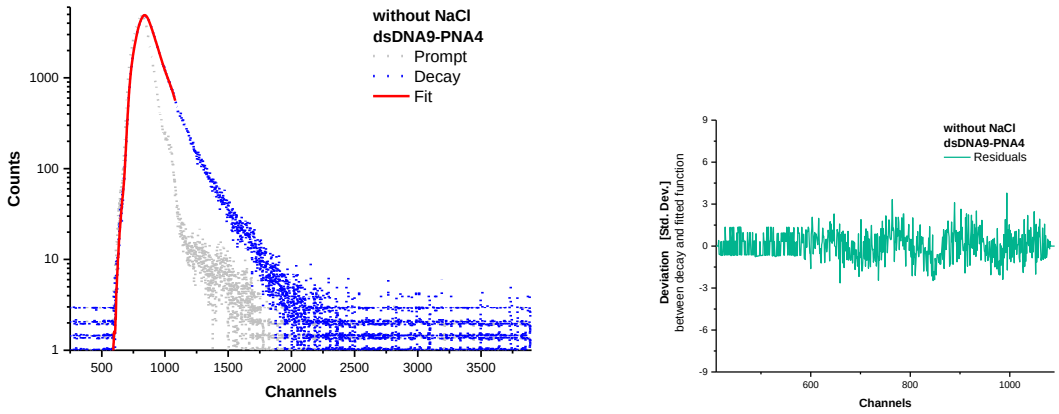
Scheme S162: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion compare solution with DNA20 and 1.5 equivalents PNA4 in presence of NaCl.



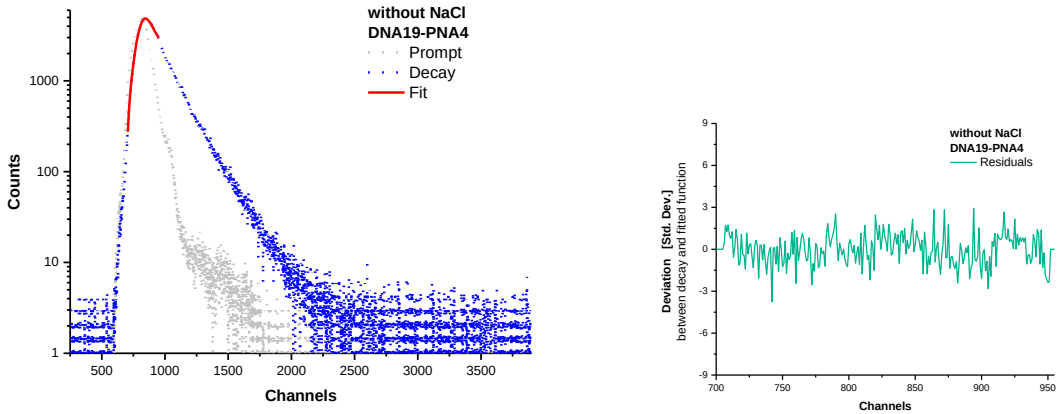
Scheme S163: Fluorescence lifetime decay of donor dye 14 (left) and residuals (right) of strand invasion PNA4-DNA21 in absence of NaCl.



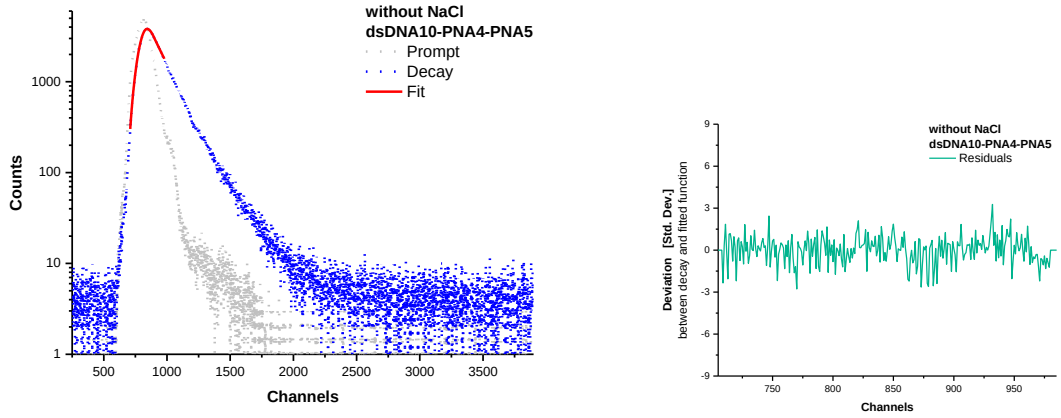
Scheme S164: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion complex dsDNA9 with 1.5 equivalents PNA4/PNA5 in absence of NaCl.



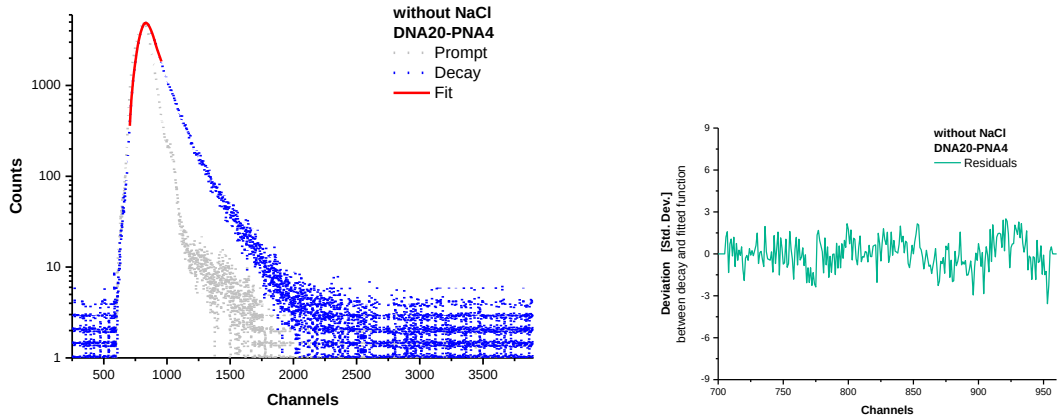
Scheme S165: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion complex dsDNA9 with 1.5 equivalents PNA4 in absence of NaCl.



Scheme S166: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion compare solution with DNA19 and 1.5 equivalents PNA4 in absence of NaCl.



Scheme S167: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion complex dsDNA10 with 1.5 equivalents PNA4/PNA5 in absence of NaCl.

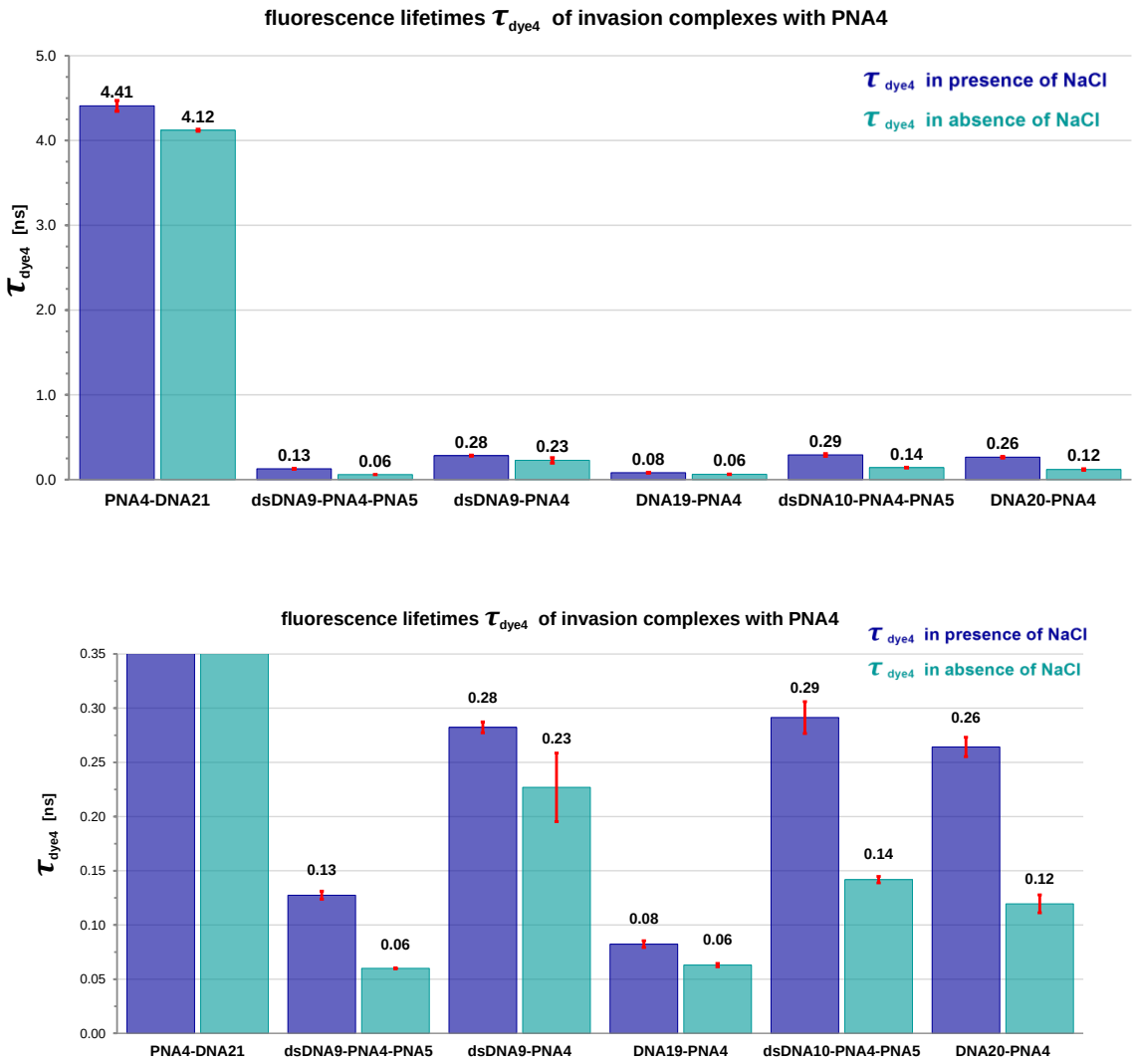


Scheme S168: Fluorescence lifetime decay of donor dye 4 (left) and residuals (right) of strand invasion compare solution with DNA20 and 1.5 equivalents PNA4 in absence of NaCl.

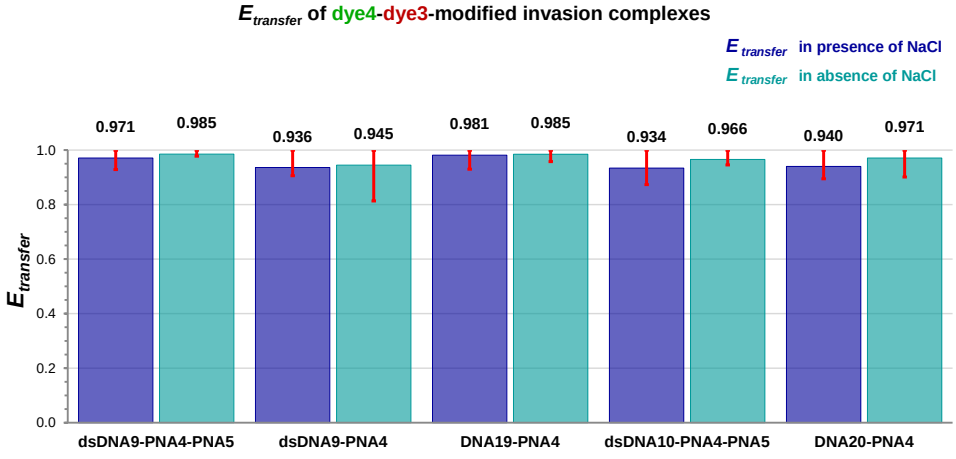
Invasion Efficiency into dsDNA by PNA4, PNA5 in presence and absence of NaCl: Δ = Standard Deviation

Conditions	Duplex / Complex	τ_{dye4} [ns]	$\Delta\tau_{dye4}$ [ns]	$\Delta\tau_{dye4}$ [%]	$E_{transfer}$	$\Delta E_{transfer}$ [%]	$\Delta E_{transfer}$	$E_{invasion}$	$\Delta E_{invasion}$ [%]	$\Delta E_{invasion}$
with NaCl	PNA4-DNA21	4.408	0.064	1.44	-	-	-	-	-	-
	dsDNA9-PNA4-PNA5	0.127	0.004	2.95	0.971	4.40	0.043	0.990	9.65	0.094
	dsDNA9-PNA4	0.282	0.005	1.79	0.936	3.23	0.030	0.954	8.49	0.082
	DNA19-PNA4	0.082	0.003	3.81	0.981	5.26	0.052	-	-	-
	dsDNA10-PNA4-PNA5	0.291	0.015	5.05	0.934	6.50	0.061	0.993	11.34	0.106
	DNA20-PNA4	0.264	0.009	3.40	0.940	4.84	0.045	-	-	-
without NaCl	PNA4-DNA21	4.121	0.011	0.28	-	-	-	-	-	-
	dsDNA9-PNA4-PNA5	0.060	0.0005	0.80	0.985	0.80	0.008	1.001	3.57	0.035
	dsDNA9-PNA4	0.227	0.032	13.94	0.945	13.94	0.132	0.960	16.70	0.159
	DNA19-PNA4	0.063	0.002	2.48	0.985	2.76	0.027	-	-	-
	dsDNA10-PNA4-PNA5	0.142	0.003	2.10	0.966	2.10	0.020	0.994	9.28	0.090
	DNA20-PNA4	0.119	0.008	6.90	0.971	7.18	0.070	-	-	-

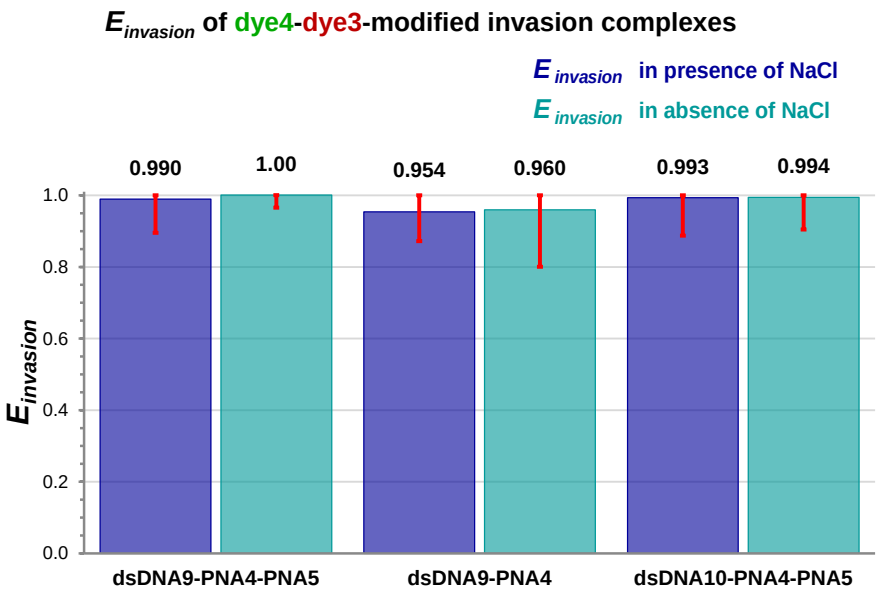
Table S4: Fluorescence lifetimes, energy transfer efficiencies and invasion efficiencies of strand invasion complexes with PNA4/PNA5.



Scheme 169: Fluorescence lifetimes of strand invasion complexes with PNA4/PNA5 (top) and magnification (bottom).



Scheme 170: Energy transfer efficiencies of strand invasion complexes with PNA4/PNA5.



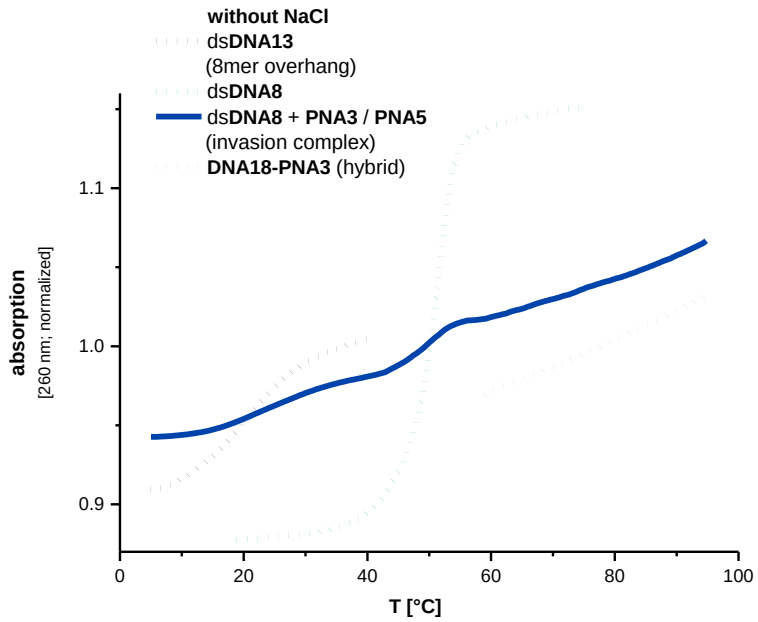
Scheme 171: Invasion efficiencies of strand invasion complexes with PNA4/PNA5.

6.5 Melting temperatures:

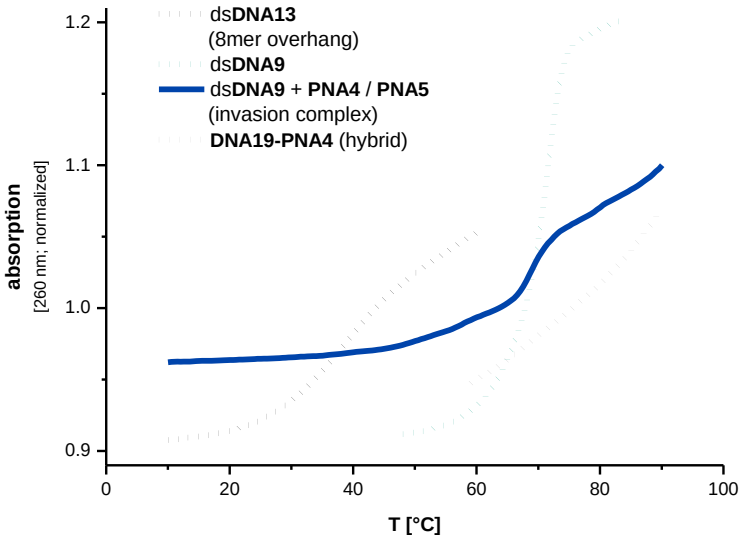
λ = 260 nm; 5 - 95 °C; interval: 0.5 °C/min; 2.5 µM PNA/DNA in 10 mM NaP_i-buffer (pH = 7.0) in absence or presence of 250 mM NaCl, respectively.

duplex (1:1)	mp [°C] with 250 mM NaCl	mp [°C] without NaCl
dsDNA13	39.0	19.1
dsDNA7	68.7	49.4
dsDNA8	69.0	50.4
dsDNA9	69.7	51.3
dsDNA10	70.5	50.7
DNA16-DNA15	70.4	48.7
PNA3-DNA17	> 90	> 90
PNA3-DNA18	> 90	> 90
PNA3-DNA16	> 90	82.8
PNA4-DNA19	> 90	> 90
PNA4-DNA20	> 90	> 90
PNA4-DNA16	> 90	88.6
DNA14-DNA16	36.5	17.1
PNA3-PNA5	< 10	< 10
PNA4-PNA5	< 10	< 10
DNA14-PNA5	> 90	> 90
PNA3-DNA21	> 90	> 90
PNA4-DNA21	> 90	> 90
DNA14-DNA21	36.3	15.5

Table S5: Melting temperatures of strand invasion.



Scheme S172: Comparison of normalized absorption trend of melting temperature determination of invasion complex dsDNA8 with 1.0 equivalent PNA3-PNA5 (blue), dsDNA8 (light green), 8mer dsDNA13 (black) and DNA18/PNA3-hybrid (light grey) in absence of NaCl, respectively.



Scheme S173: Comparison of normalized absorption trend of melting temperature determination of invasion complex dsDNA9 with 1.0 equivalent PNA4-PNA5 (blue), dsDNA9 (light green), 8mer dsDNA13 (black) and DNA19/PNA4-hybrid (light grey) in presence of NaCl, respectively.

7. Preparation and purification of PNA:

7.1: Synthesis of PNA:

The APC-modified acpcPNA was synthesized manually on Tentagel S-RAM resin from the respective Fmoc-protected monomers at a 0.5 µmol scale according to the previously published protocol.[5],[6] 5 Lysine residues were always included at the C-termini to improve water solubility and cell permeability. After completion of the synthesis, the N-terminal Fmoc group was removed and the free amino group was capped by acetylation. The trifluoroacetyl protecting group of the APC residue and the nucleobase side chain protecting groups were removed by treatment with 1:1 aqueous ammonia-dioxane (1:1) at 60 °C for 16 h.

Reductive alkylation of APC-spacer:[7]
The deprotected APC-modified acpcPNA (0.5 µmol) was treated overnight with the aldehyde (4-Pentin-1-al, 15 µmol, 30 equivalents) in the presence of $NaBH_3CN$ (30 µmol, 60 equivalents) and acetic acid (30 µmol, 60 equivalents) in 100 µL methanol at room temperature. Afterwards the solid support was washed with methanol and dried under a stream of nitrogen.

7.2 "Click" modification of PNA:

37.5 µL of an aqueous sodium ascorbate solution (0.4 mM in water), 51 µL tris-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (0.1 mM DMSO / t-butanol = 3:1), 25.5 µL of a solution of tetrakis(acetonitrile)copper(I)hexafluorophosphate (0.1 mM DMSO / t-butanol = 3:1), 95 µL acetonitrile and finally 171 µL of azide (0.01 M, DMSO / t-butanol = 3:1) were mixed thoroughly. 0.5 µmol PNA (on solid support) was soaked with this reaction mixture and kept 3 h at 60 °C. The reaction solution was mixed every 30 min to ensure complete reaction. After cooling to room temperature the solid support was washed 3 times with acetonitrile and methanol, respectively. Afterwards the dried PNA was cleaved with TFA (3 times with approximately 500 µL TFA) and the combined TFA washing was evaporated under a stream of nitrogen and the acpcPNA was precipitated by the addition of diethyl ether. After washing with more diethyl ether and air-dried, the crude acpcPNA was solubilized in 250 µL water for HPLC-purification.

7.3 HPLC-purification and MALDI Mass spectra of PNA1 to PNA6:

PNA strands were purified by HPLC Reversed Phase *Supelcosil™* LC-C18 column (250 x 10 mm, 5 µm) on a *Shimadzu* HPLC system (autosampler SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A) using the following conditions: A) Water + 0.1 % trifluoracetic acid; B) methanol + 0.1 % trifluoracetic acid; for gradient see Table S6; flow rate 2.0 mL/min; room temperature; UV/Vis detection at 260 nm, 385 nm for **PNA1**, **PNA2** and **PNA6**, 260 nm, 506 nm for **PNA3**, 260 nm, 459 nm for **PNA4** and 260 nm for **PNA5**.

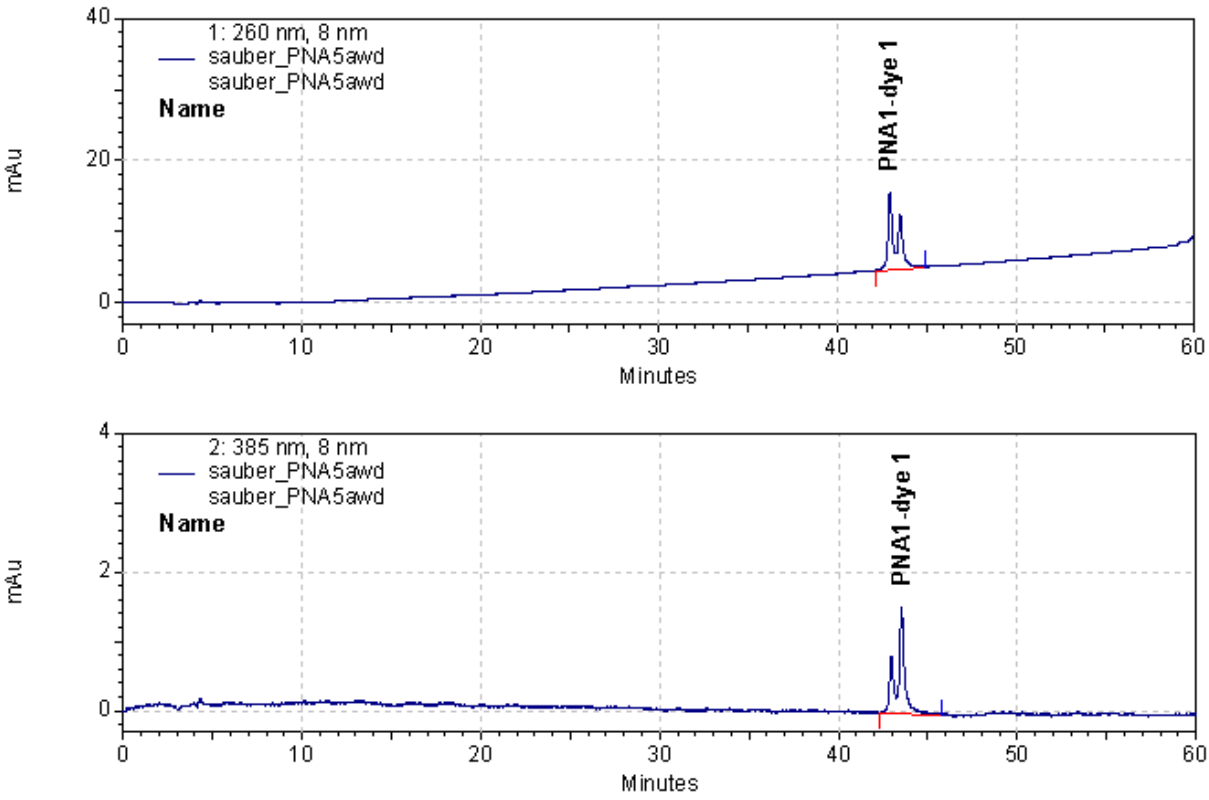
time [min]	amount of eluent B [%]
0	20
5	20
60	70
80	70
85	90
90	90
91	20
105	20

Table S6: HPLC-conditions for semi-preparative purification of PNA by reversed phase HPLC.

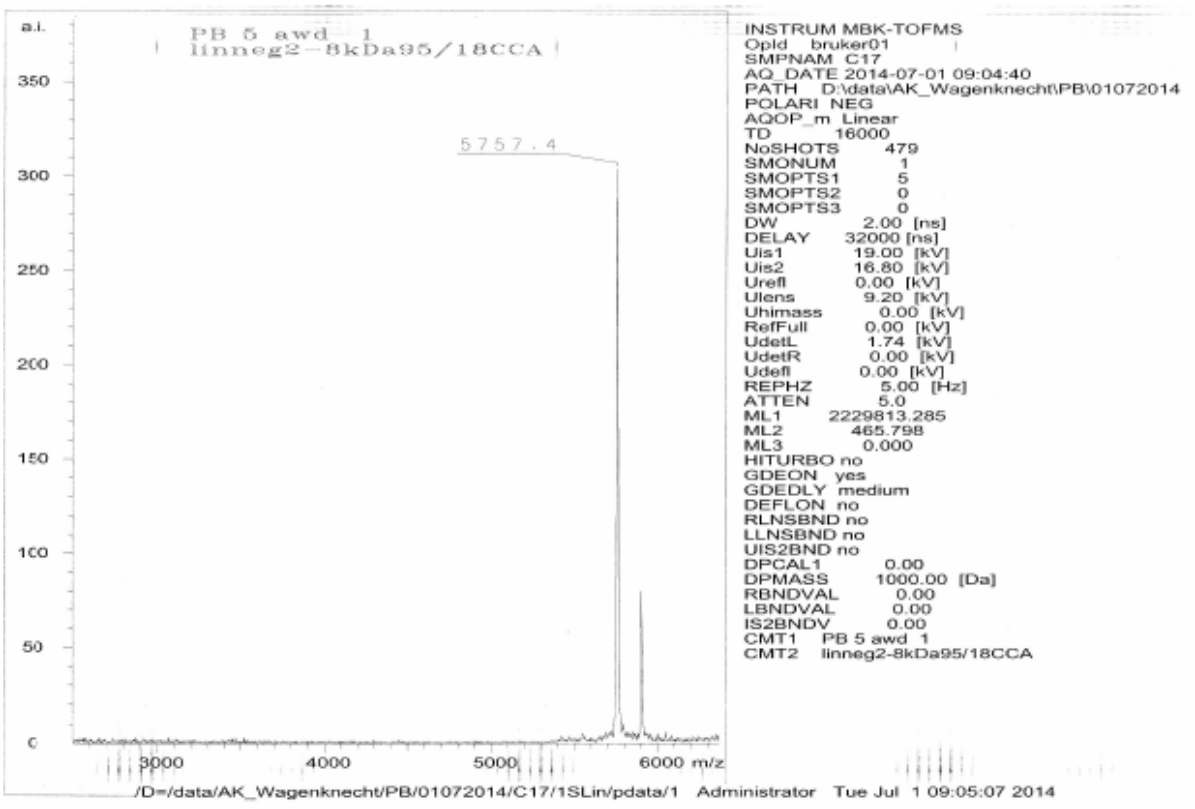
Analytical HPLC of the purified oligonucleotides were determined with Reversed Phase *Supelcosil™* LC-C18 column (250 x 4.5 mm, 5 µm) using the following conditions: A) Water + 0.1 % trifluoracetic acid; B) methanol + 0.1 % trifluoracetic acid; for gradient see Table S7; flow rate 0.8 mL/min; room temperature; UV/Vis detection at 260 nm, 385 nm for **PNA1**, **PNA2** and **PNA6**, 260 nm, 506 nm for **PNA3**, 260 nm, 459 nm for **PNA4**.

time [min]	amount of eluent B [%]
0	10
60	70
80	70
81	90
95	90
96	10
110	10

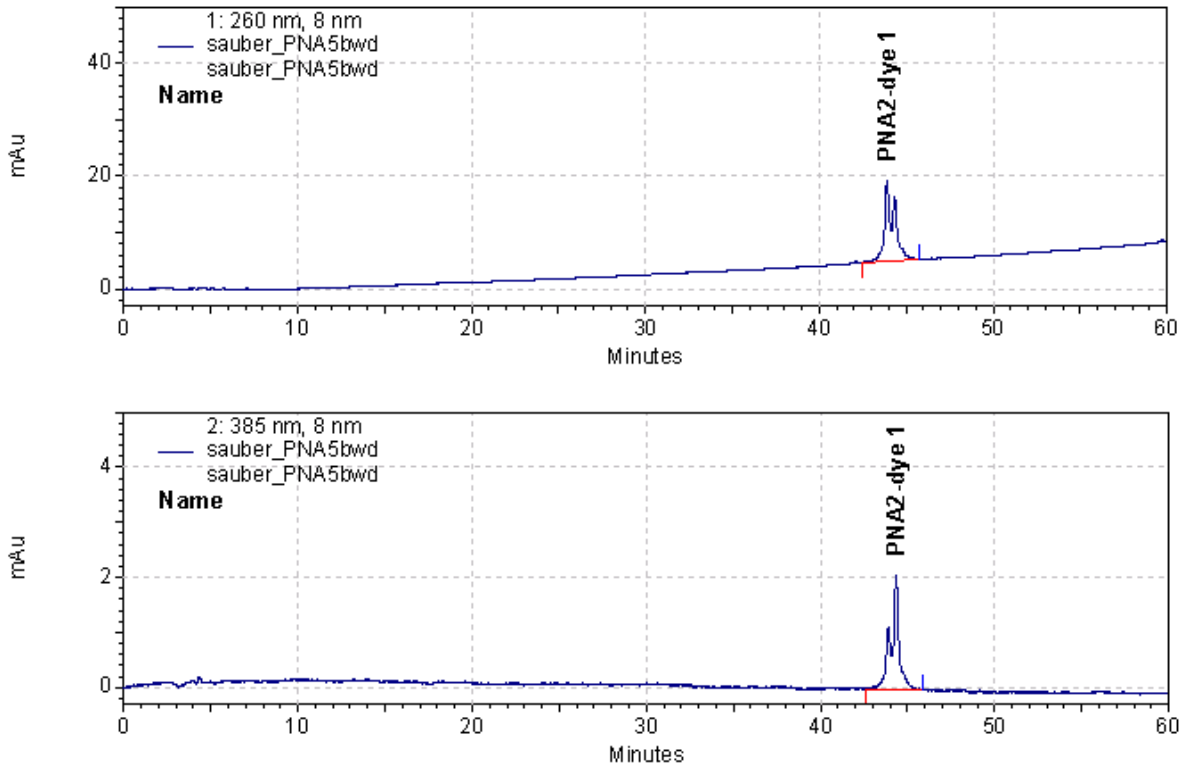
Table S7: HPLC-conditions for analytical analysis of the purified PNA.



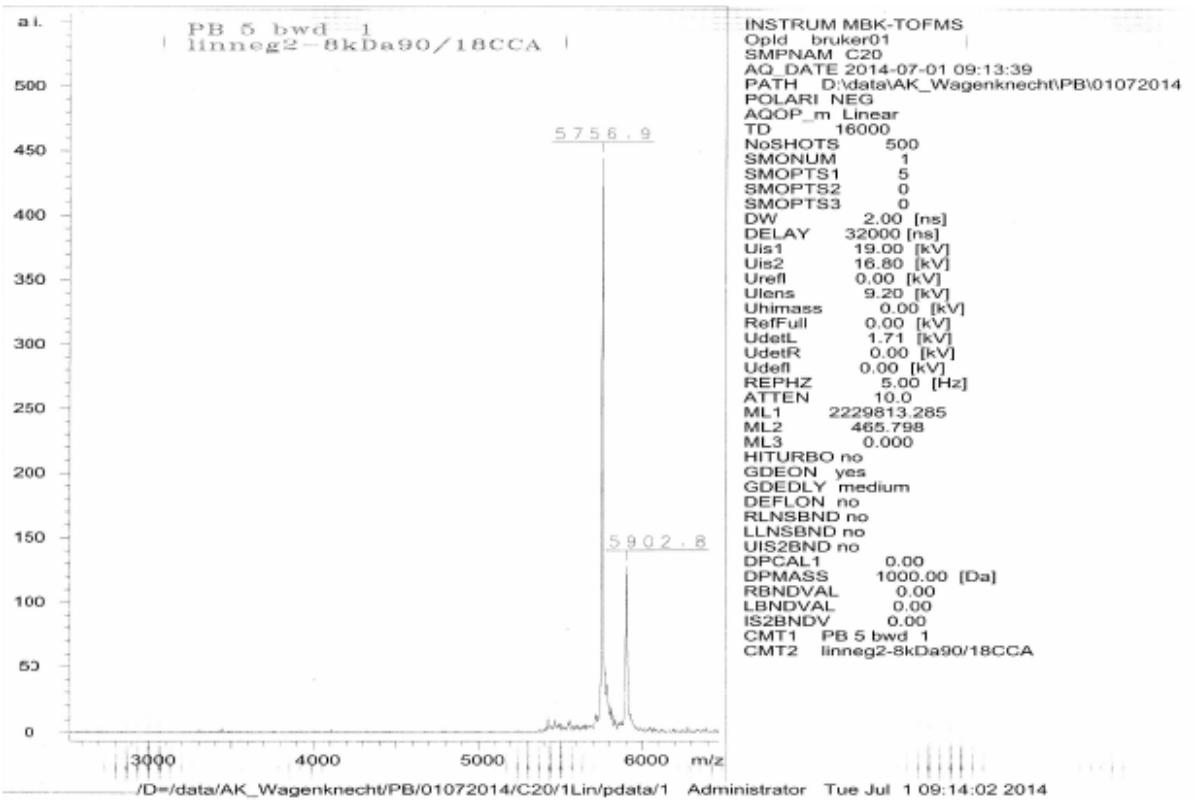
Scheme S174: HPLC-chromatogram of purified **PNA1**.



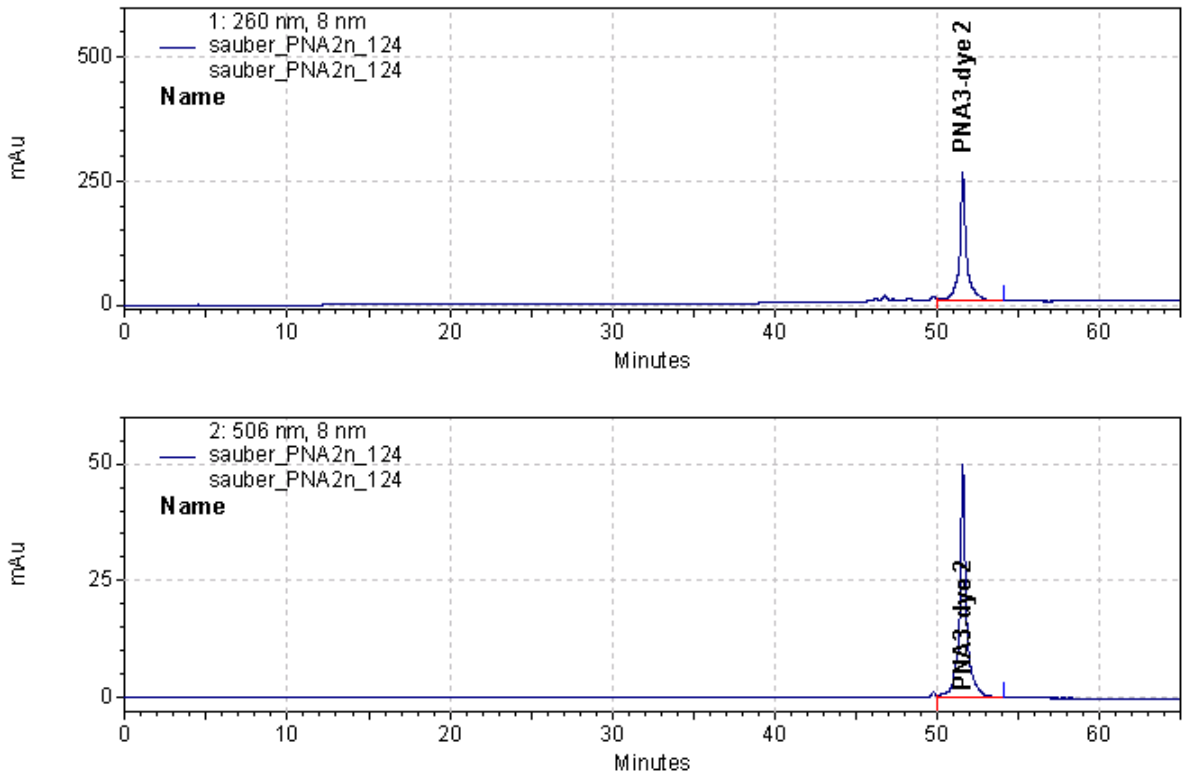
Scheme S175: MALDI spectra of **PNA1**, calculated: 5739.04 g/mol, found: 5757.4 g/mol ($+OH^-$). The mass 5903 g/mol represents residual Benzoyl-protected product ($+OH^-$, + Na^+).



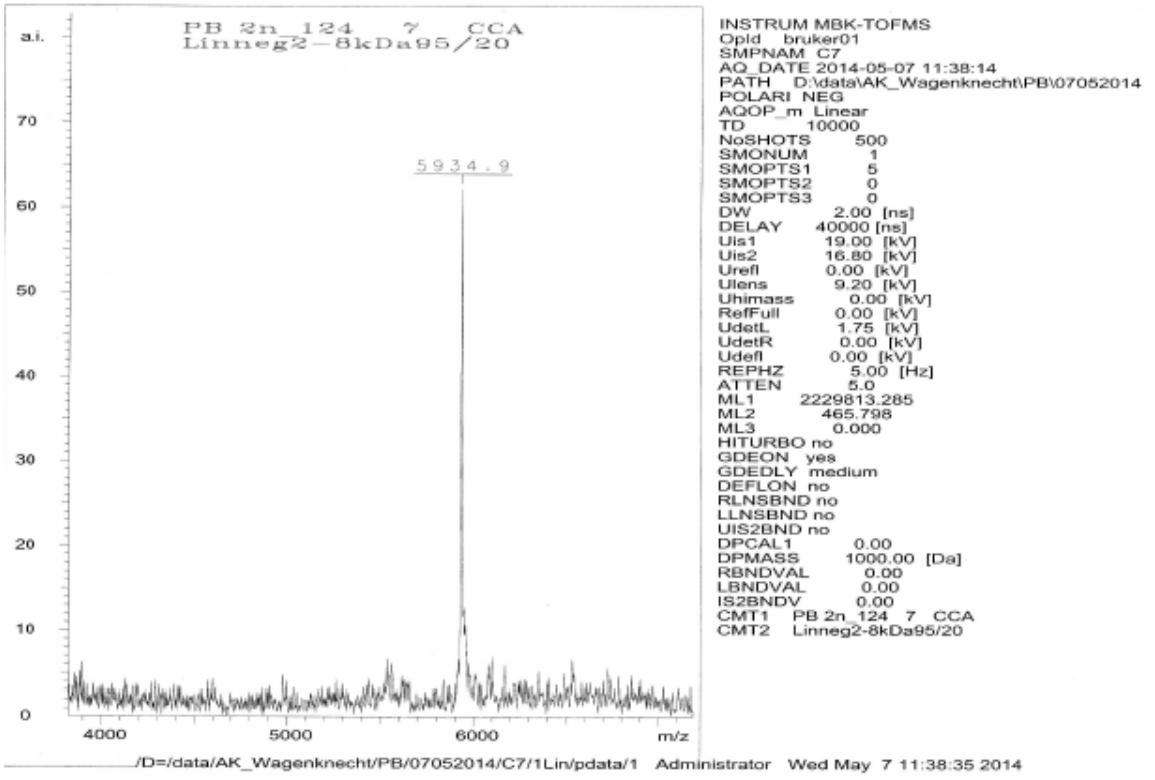
Scheme S176: HPLC-chromatogram of purified **PNA2**.



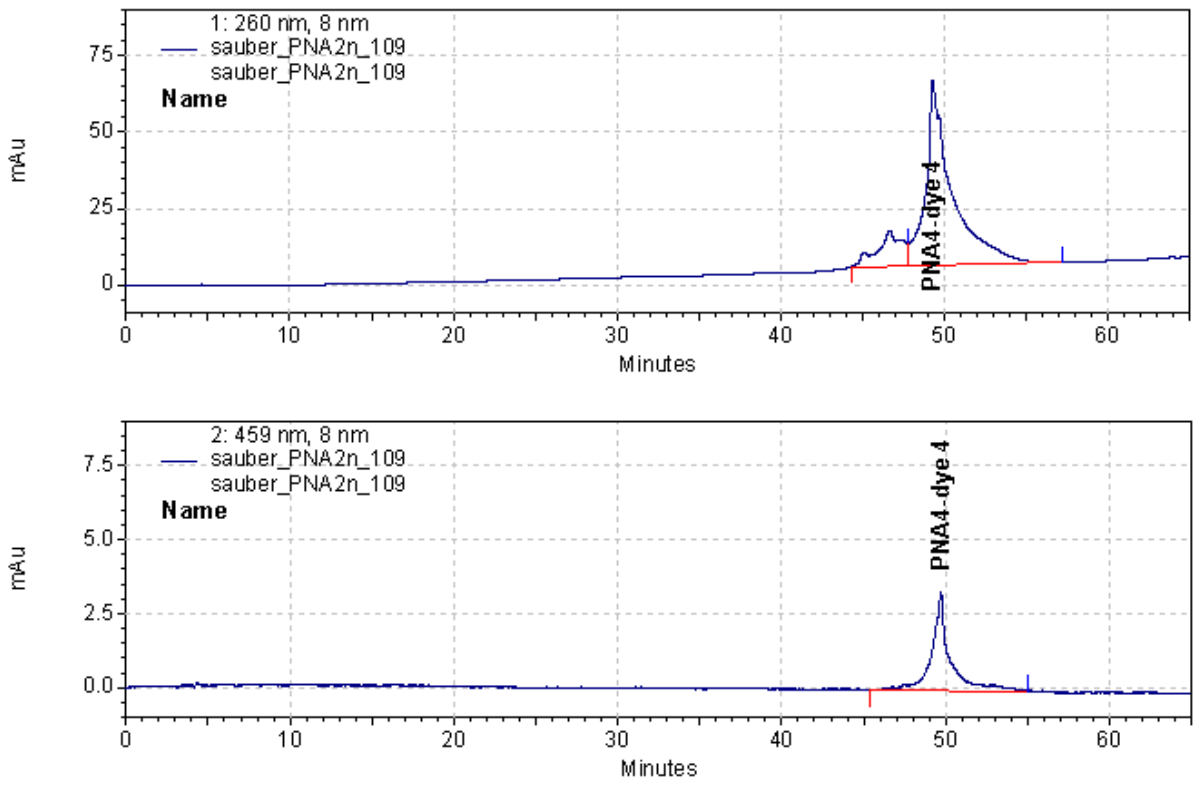
Scheme S177: MALDI spectra of **PNA2**, calculated: 5739.04 g/mol, found: 5756.9 g/mol ($+OH^-$). The mass 5903 g/mol represents residual Benzoyl-protected product ($+OH^-$, $+ Na^+$).



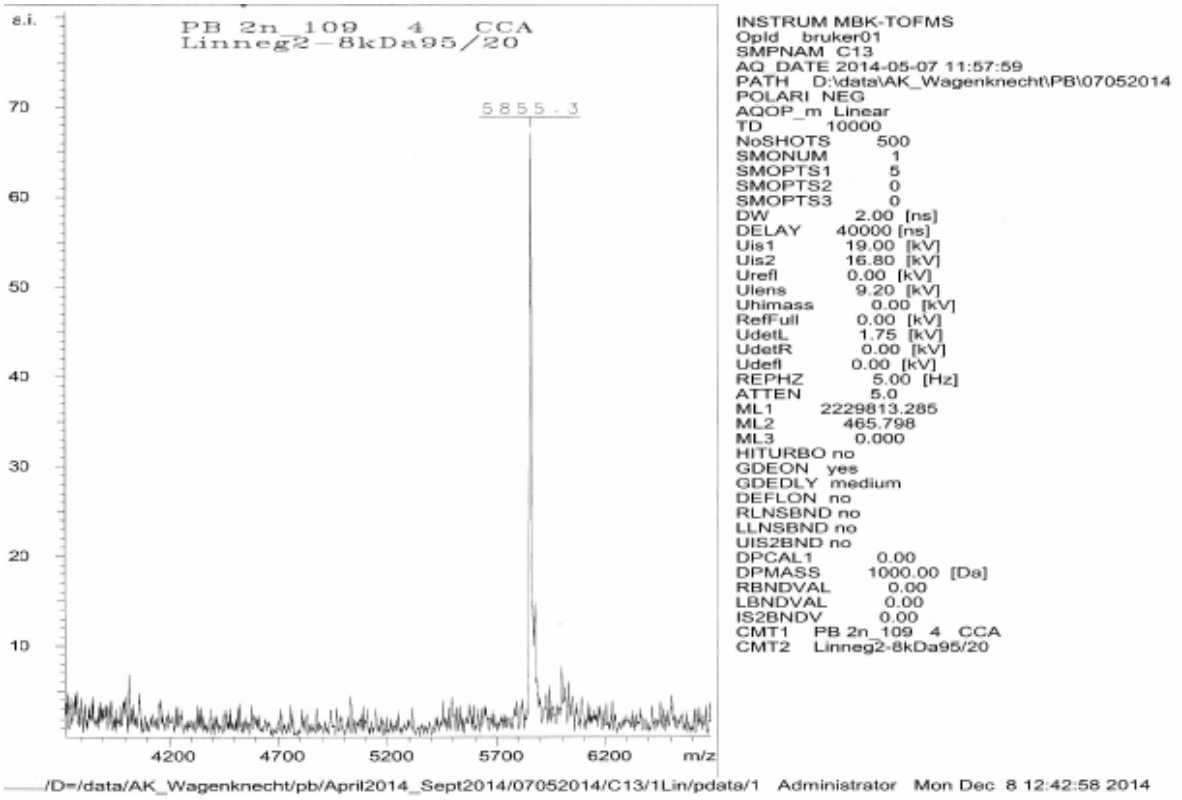
Scheme S178: HPLC-chromatogram of purified **PNA3**.



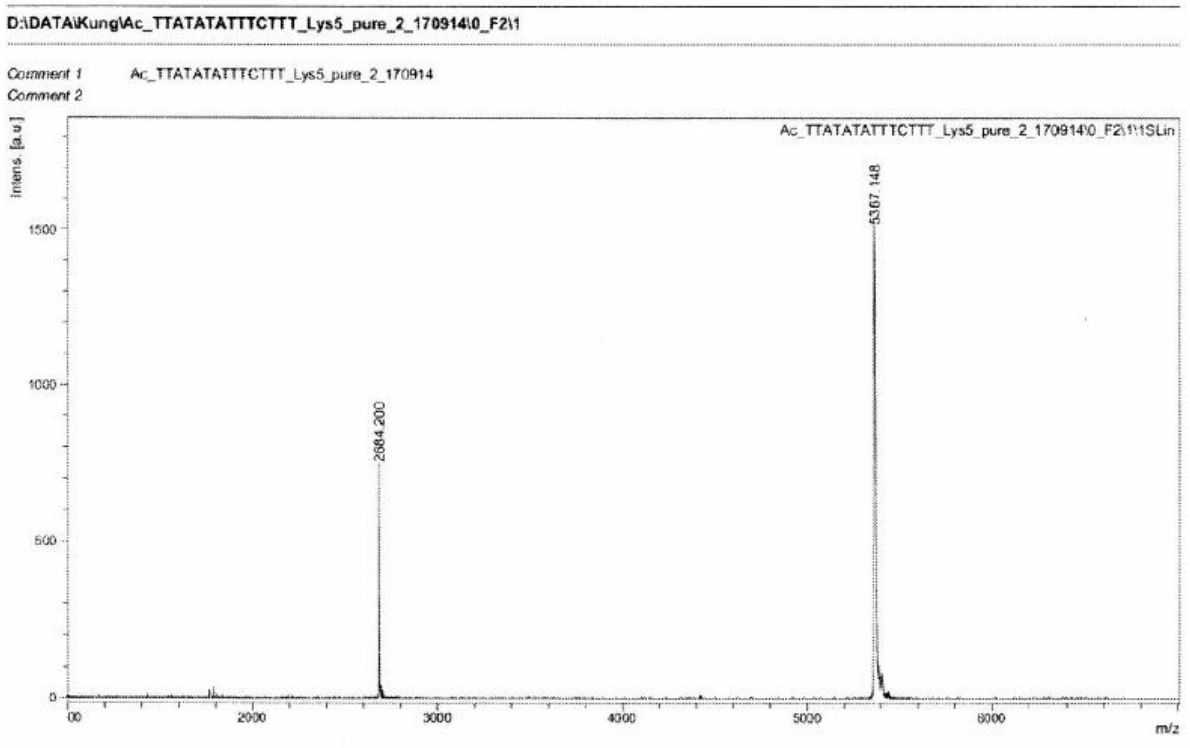
Scheme S179: MALDI spectra of **PNA3**, calculated: 5917.22 g/mol, found: 5934.9 g/mol ($+OH^-$).



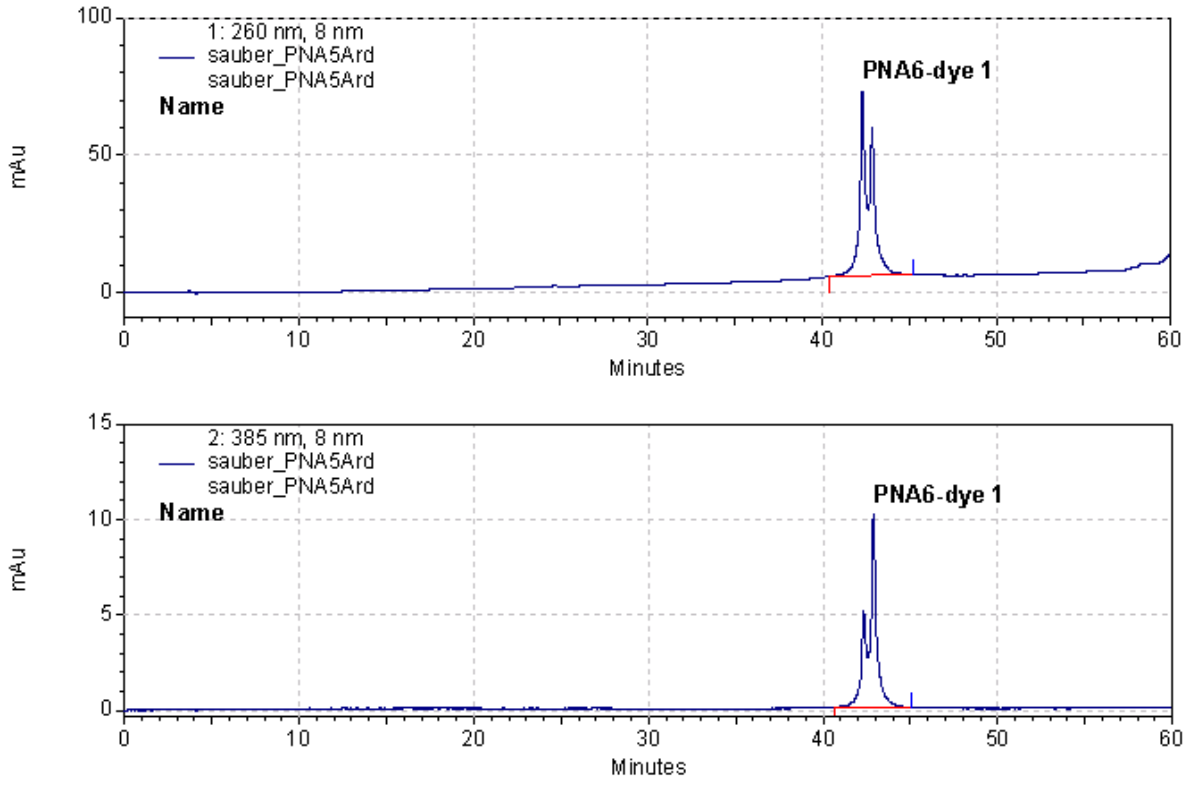
Scheme S180: HPLC-chromatogram of purified **PNA4**.



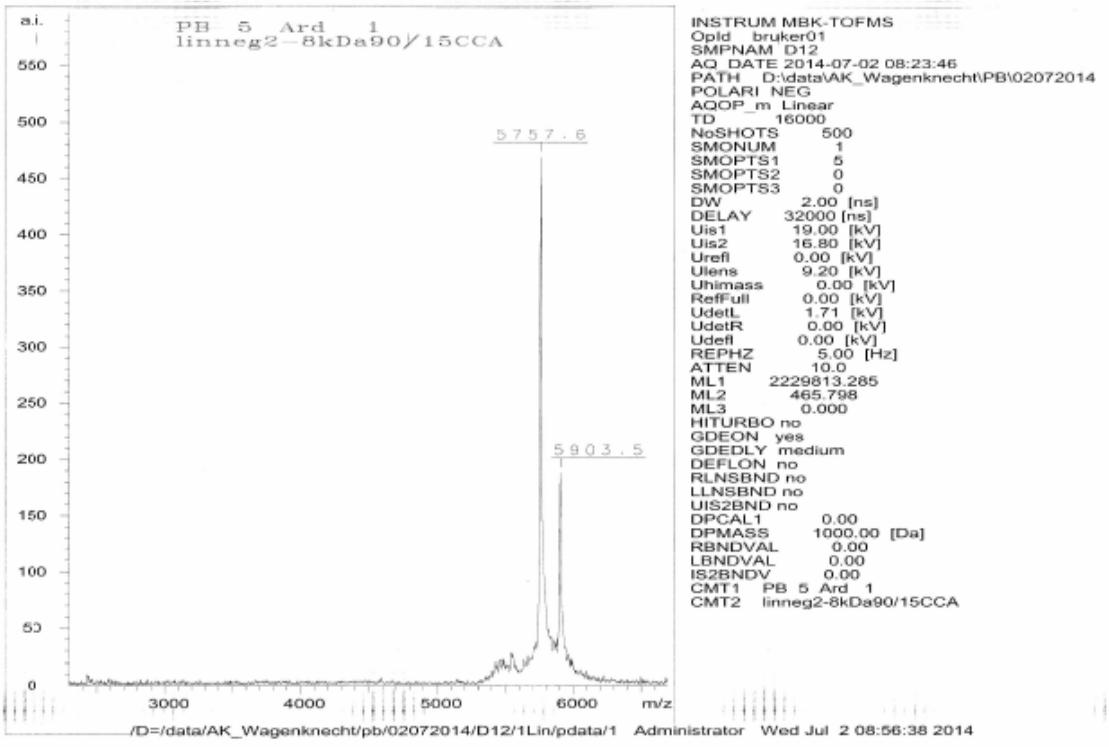
Scheme S181: MALDI spectra of **PNA4**, calculated: 5837.13 g/mol, found: 5855.3 g/mol ($+OH^-$).



Scheme S182: MALDI spectra of **PNA5**, calculated: 5365.9 g/mol, found: 5367.1 g/mol.



Scheme S183: HPLC-chromatogram of purified **PNA6**.



Scheme S184: MALDI spectra of **PNA6**, calculated: 5739.04 g/mol, found: 5757.6 g/mol ($+OH^-$). The mass 5903 g/mol represents residual Benzoyl-protected product ($+OH^-$, $+ Na^+$).

8. Preparation and purification of DNA:

8.1 Synthesis of DNA:

Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. Reagents and controlled pore glass (CPG) (1 µmol) were purchased from ABI and Glen Research. 2´propargyl-adenosine (cA) and 2´propargyl-uridine (cU) were purchased from ChemGenes. DNA synthesis was performed using standard coupling conditions since the cA and cU building blocks contain a phosphoramidite group at the 3´-position and a DMT group at the 5´-position. The concentration of the artificial building blocks was 0.1 mol/L (in acetonitrile). After preparation, the oligonucleotides were cleaved from the resin and deprotected by treatment with conc. NH_4OH at 55 °C for 18 h.

8.2 "Click" modification of DNA:

25 µL of an aqueous sodium ascorbate solution (0.1 mM in water), 34 µL tris-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (0.1 mM DMSO / t-butanol = 3 : 1), 17 µL of a solution of tetrakis(acetonitrile)copper(I)hexafluorophosphate (0.1 mM DMSO / t-butanol = 3 / 1), 35 µL acetonitrile and finally 114 µL of azide (0.01 M, DMSO / t-butanol = 3 : 1) were added to the 2´-propargyl-uridine modified DNA (solubilized in 50 µL water). The reaction solution was mixed thoroughly and kept 1.5 h at 60 °C. The reaction solution was mixed every 30 min to ensure complete reaction. After cooling to room temperature the mixture was diluted with 150 µL Na_2EDTA (40 mM in water), 450 µL sodium acetate (300 mM in water) and 13 mL ethanol (100%) and stored for 18 h at -20 °C. Afterwards the suspension was centrifuged (4000 rpm, 10 min) and the supernatant removed. The pellet was solubilized in 250 µL water for HPLC-purification.

8.3 HPLC-purification and MALDI Mass spectra of modified DNA:

The modified oligonucleotides were purified by HPLC Reversed Phase *Supelcosil™* LC-C18 column (250 x 10 mm, 5 µm) on a *Shimadzu* HPLC system (autosampler SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A) using the following conditions: A) NH_4OAc buffer (50 mM), pH = 6.5; B) acetonitrile; for gradient see Table S8; flow rate 2.5 mL/min; room temperature; UV/Vis detection at 260 nm, 506 nm for **DNA2** and **DNA4**; 260 nm, 559 nm for **DNA3**, **DNA5**, and **DNA19** and **DNA20** and 260 nm, 390 nm for **DNA17** and **DNA18**.

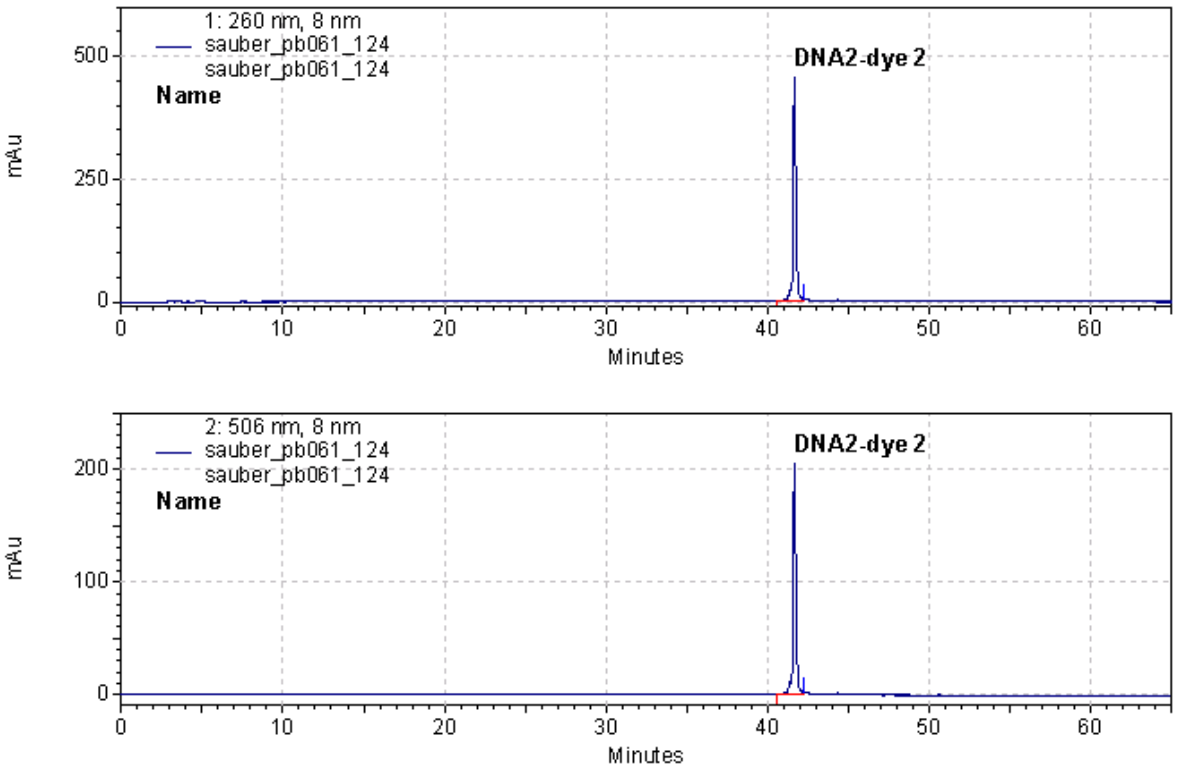
time [min]	amount of eluent B [%]
0	0
45	17
75	17
76	95
85	95
86	0
95	0

Table S8: HPLC-conditions for semi-preparative purification of DNA by reversed phase HPLC.

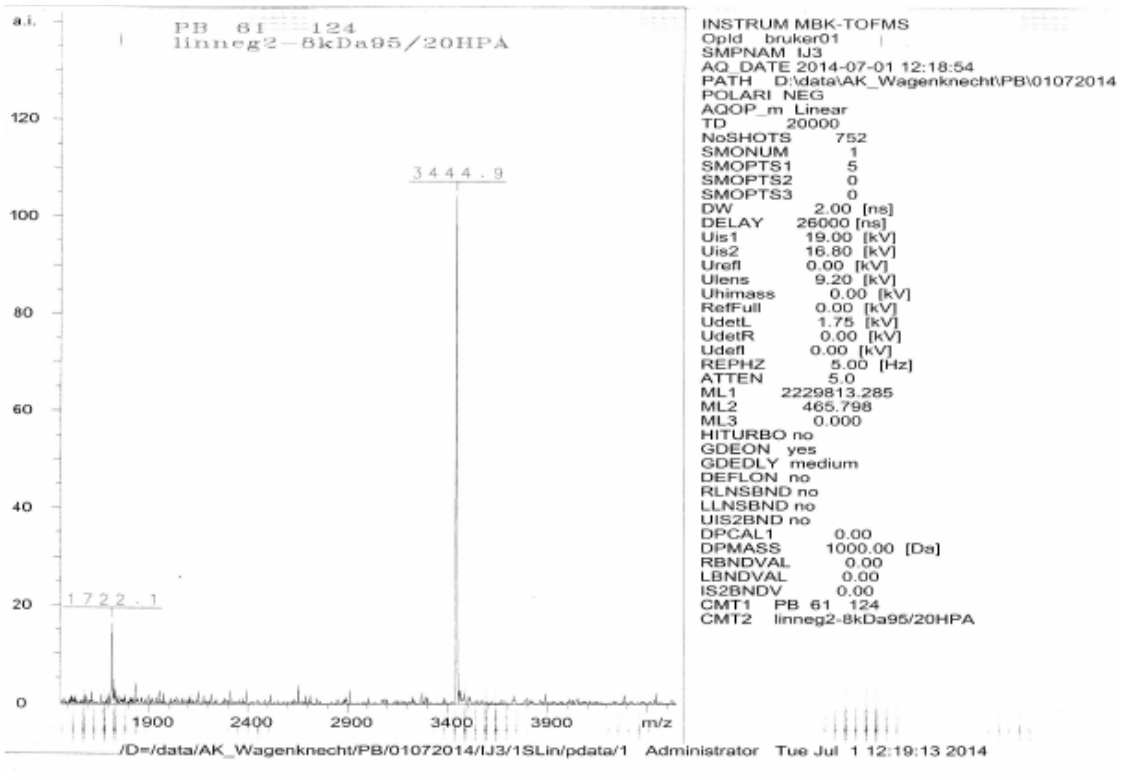
Analytical HPLC of the purified oligonucleotides were determined with Reversed Phase *Supelcosil™* LC-C18 column (250 x 4.5 mm, 5 µm) using the following conditions: A) NH_4OAc buffer (50 mM), pH = 6.5; B) acetonitrile; for gradients see Table S9; flow rate 1.0 mL/min; room temperature; UV/Vis detection at 260 nm, 506 nm for **DNA2** and **DNA4**; 260 nm, 559 nm for **DNA3**, **DNA5**, and **DNA19** and **DNA20** and 260 nm, 390 nm for **DNA17** and **DNA18**.

time [min]	amount of eluent B [%]
0	0
45	20
65	20
66	95
77	95
78	0
90	0

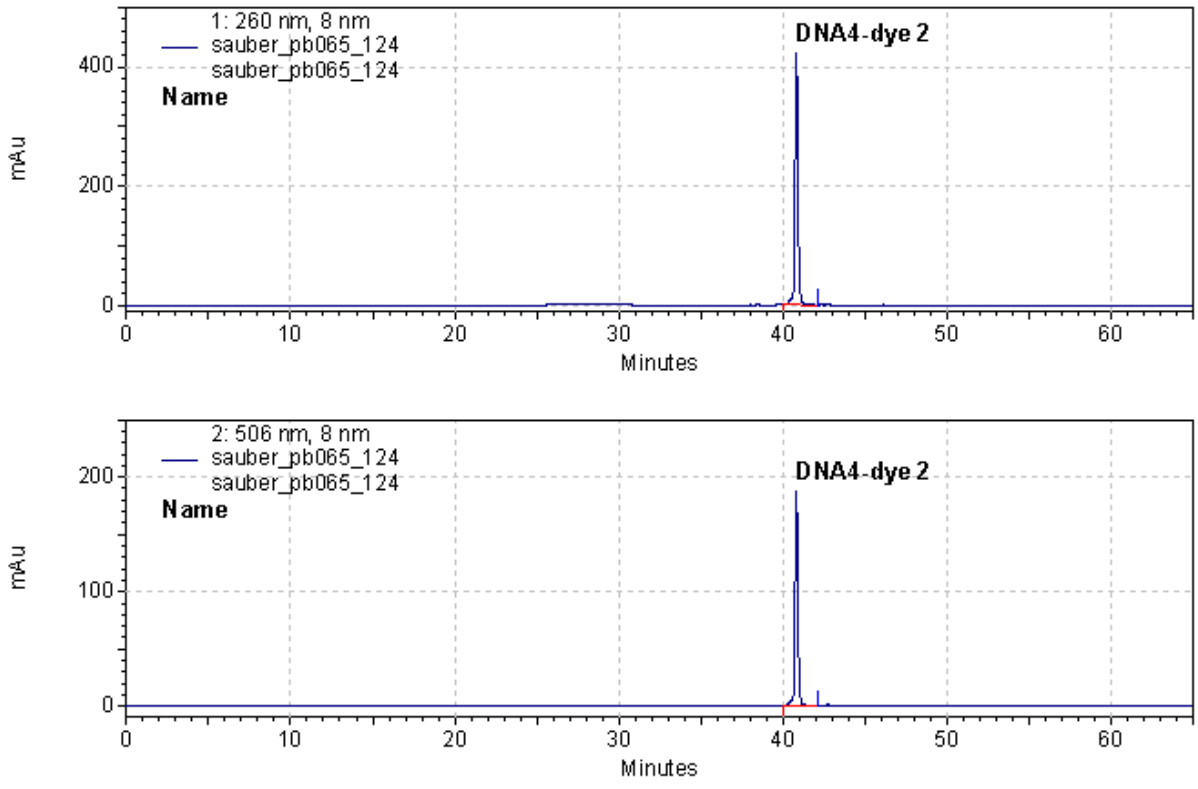
Table S9: HPLC-conditions for analytical analysis of the purified DNA.



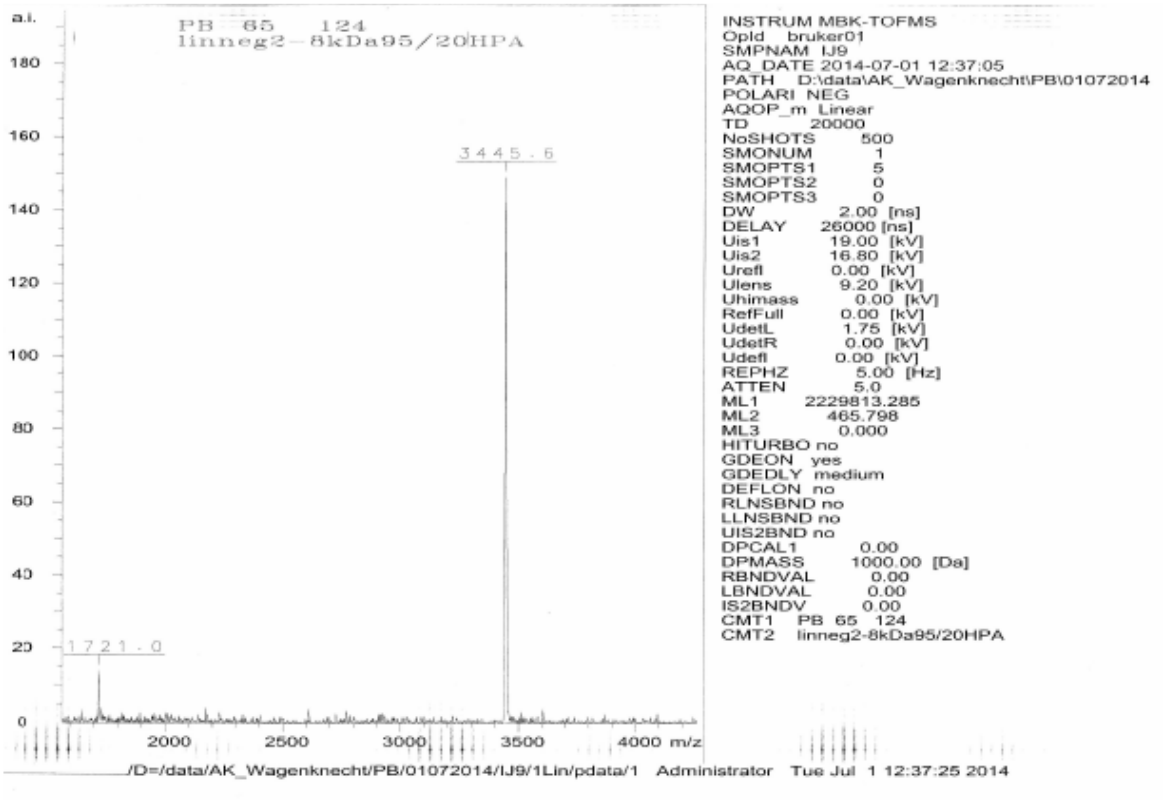
Scheme S185: HPLC-chromatogram of purified **DNA2**.



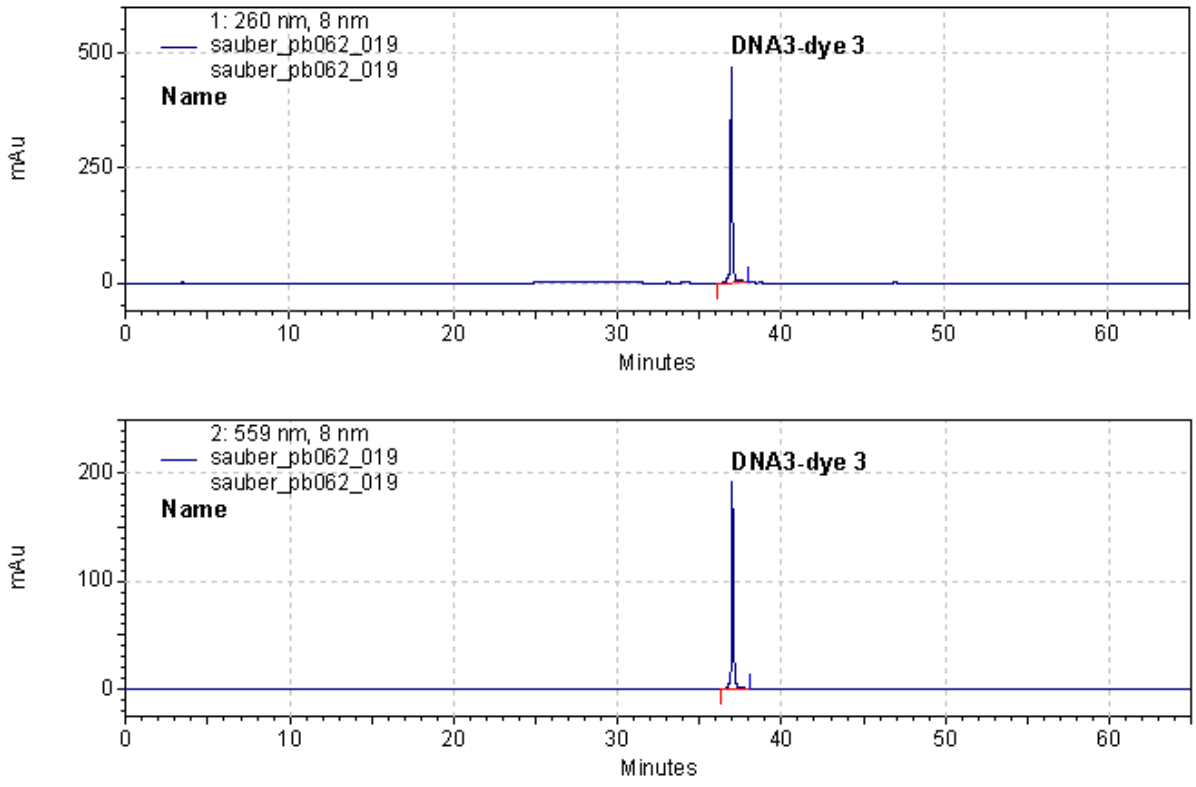
Scheme S186: MALDI spectra of **DNA2**, calculated: 3443.7 g/mol, found: 3444.9 g/mol.



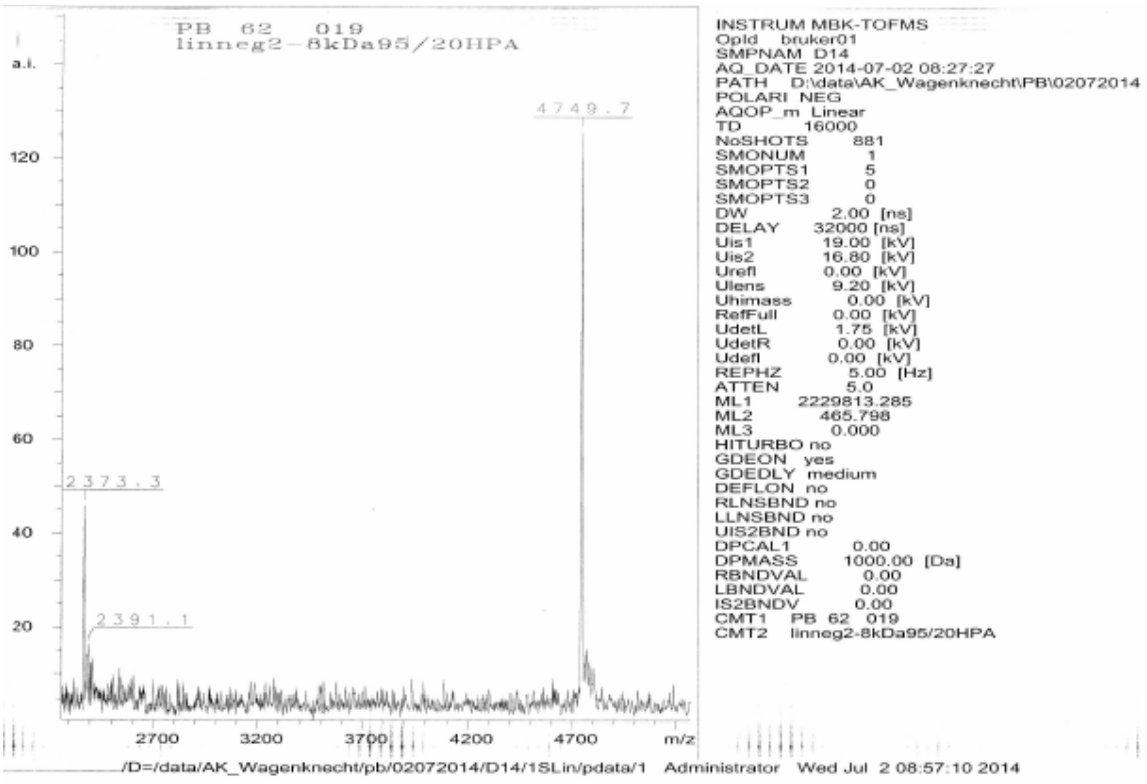
Scheme S187: HPLC-chromatogram of purified **DNA4**.



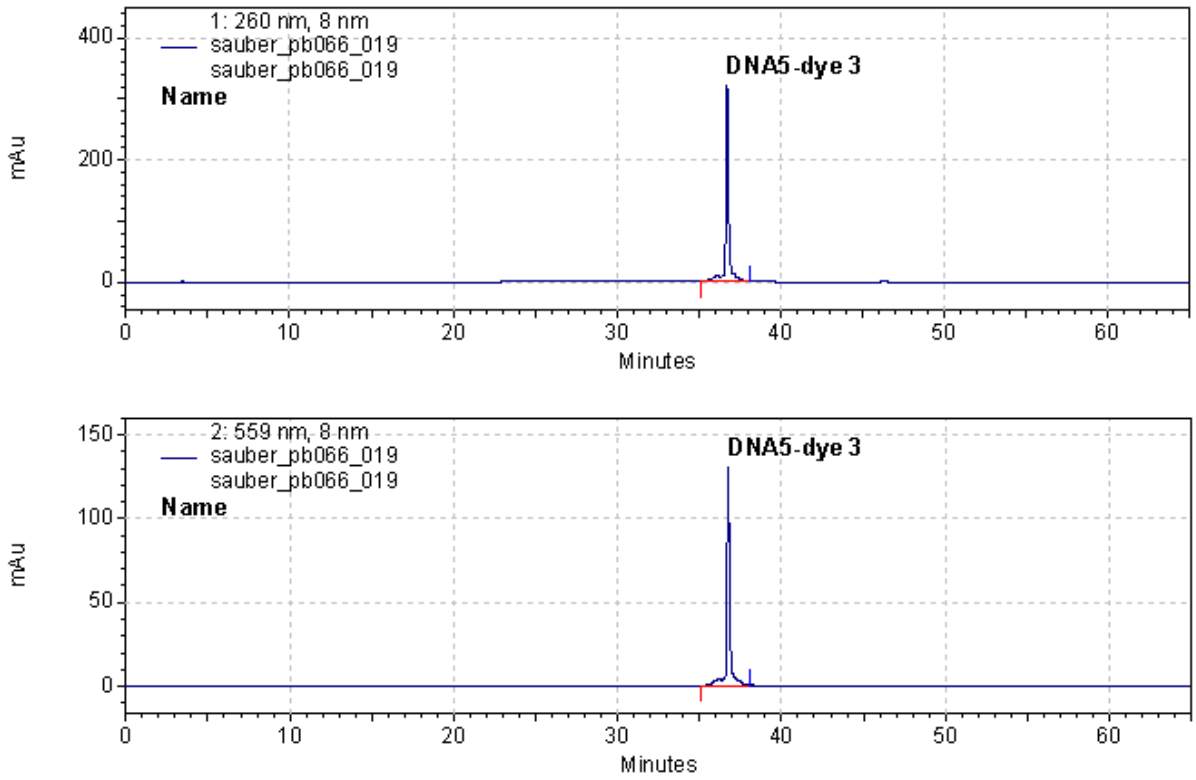
Scheme S188: MALDI spectra of **DNA4**, calculated: 3443.7 g/mol, found: 3445.6 g/mol.



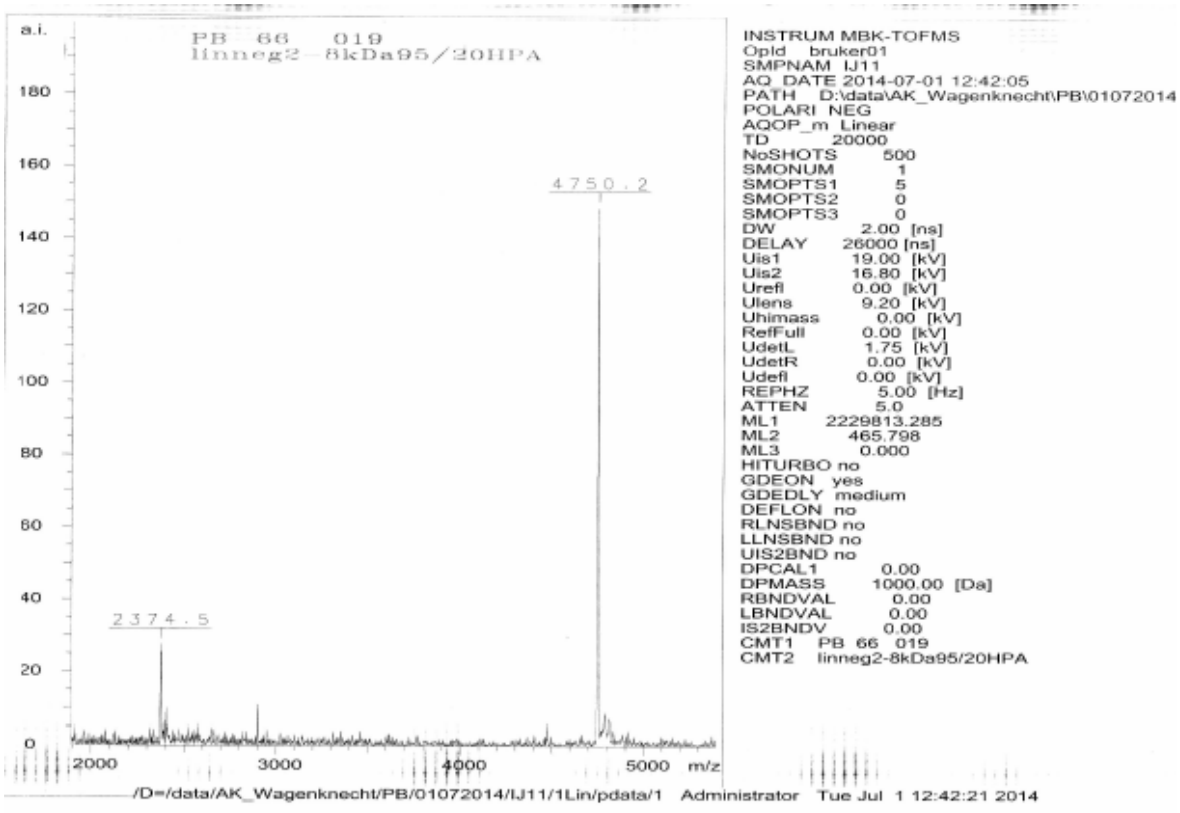
Scheme S189: HPLC-chromatogram of purified **DNA3**.



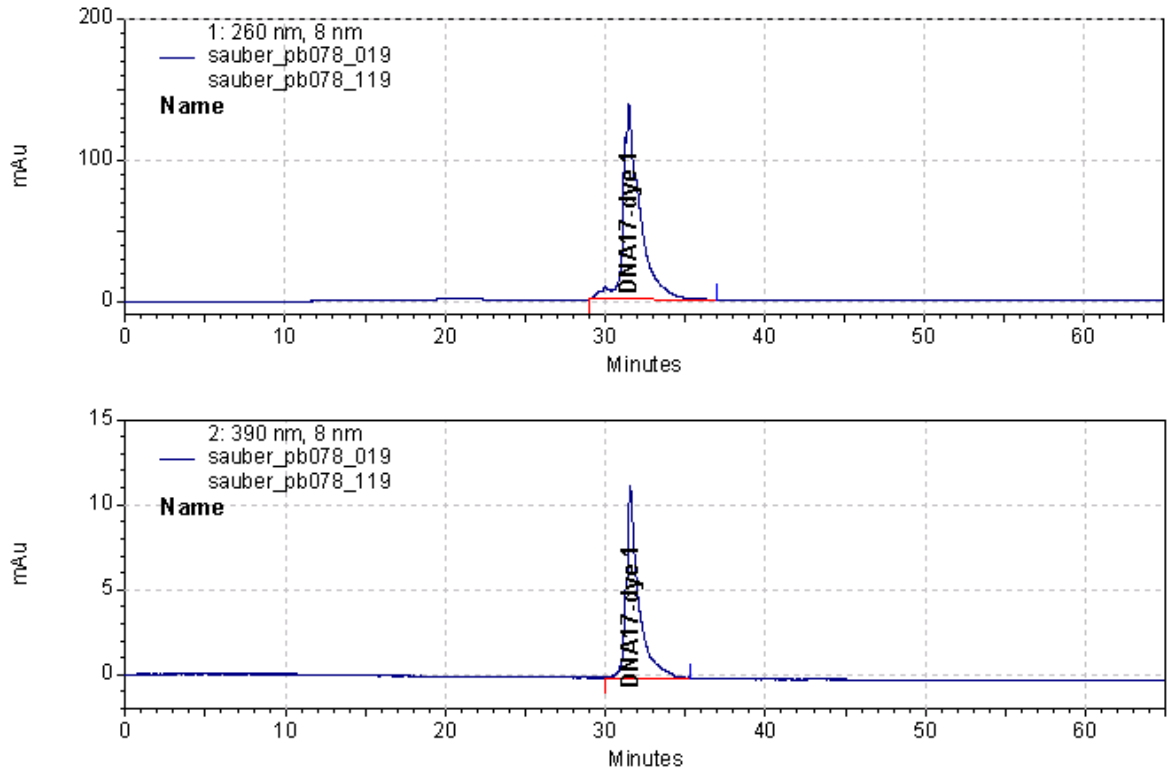
Scheme S190: MALDI spectra of **DNA3**, calculated: 4718.9 g/mol, found: 4749.7 g/mol ($+K^+$).



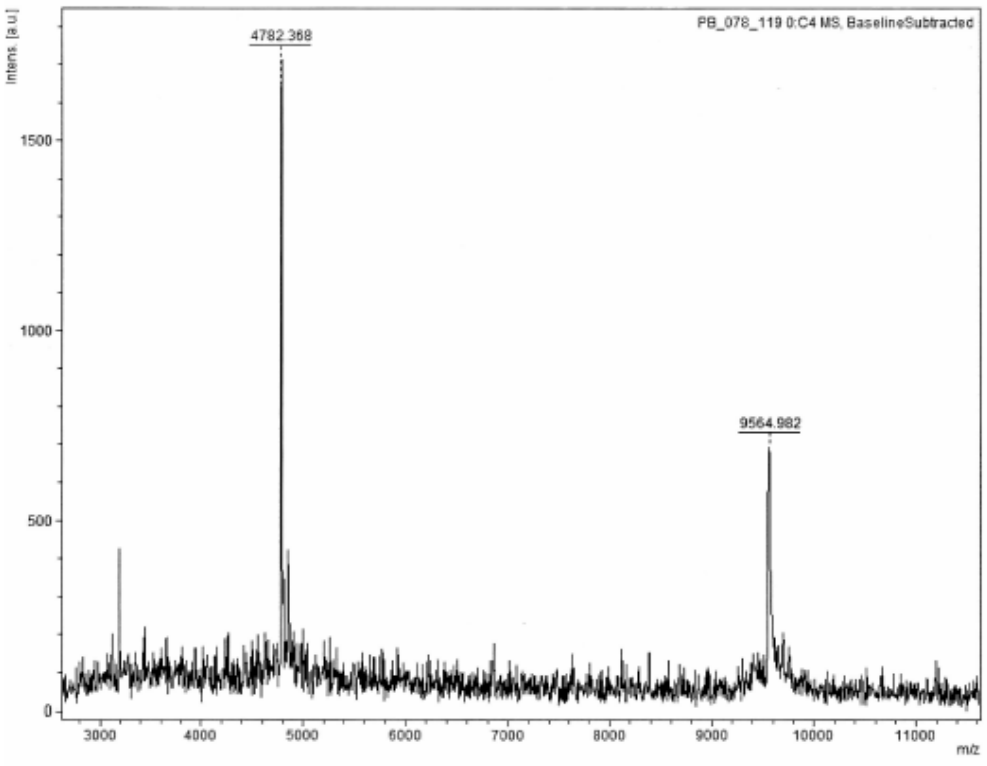
Scheme S191: HPLC-chromatogram of purified **DNA5**.



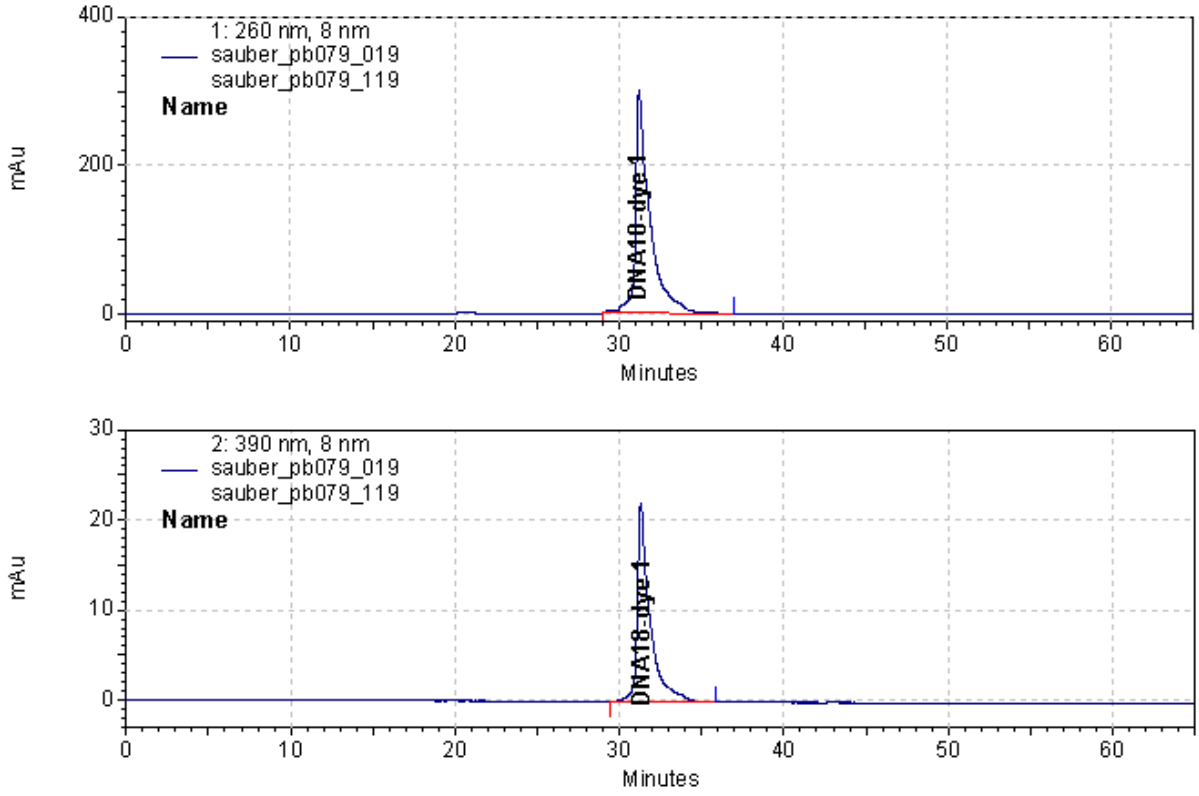
Scheme S192: MALDI spectra of **DNA5**, calculated: 4718.9 g/mol, found: 4750.2 g/mol ($+K^+$).



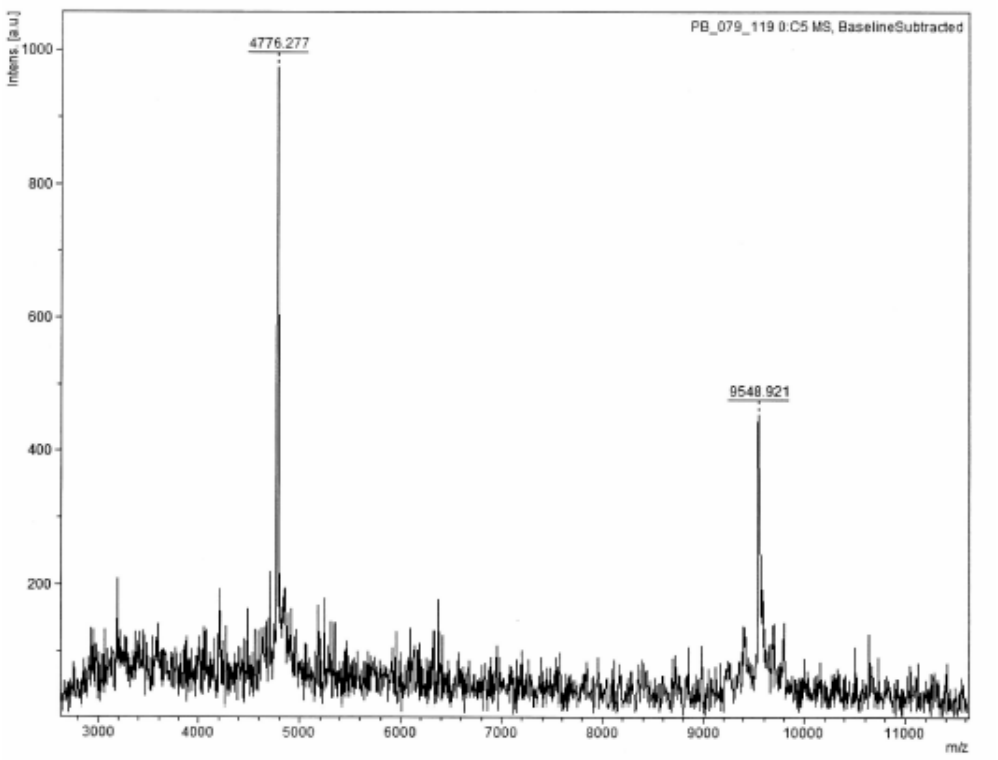
Scheme S193: HPLC-chromatogram of purified **DNA17**.



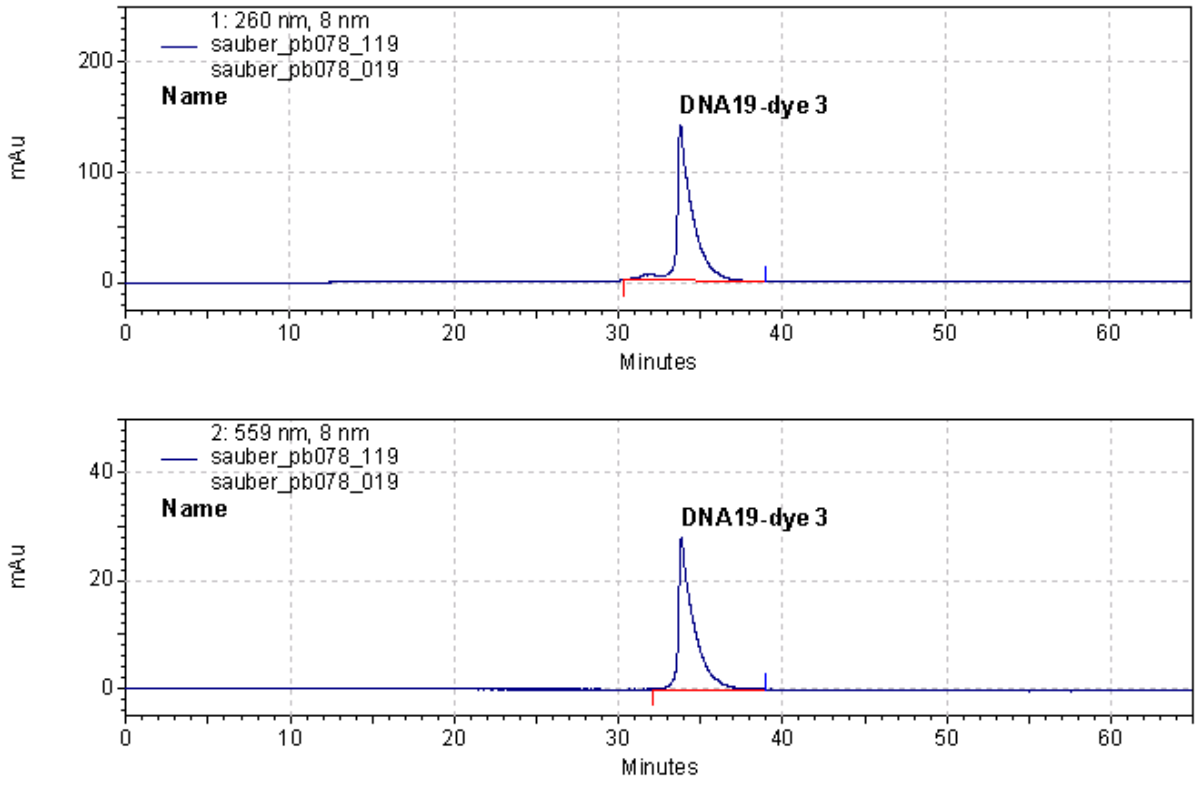
Scheme S194: MALDI spectra of **DNA17**, calculated: 9561.7 g/mol, found: 9565.0 g/mol.



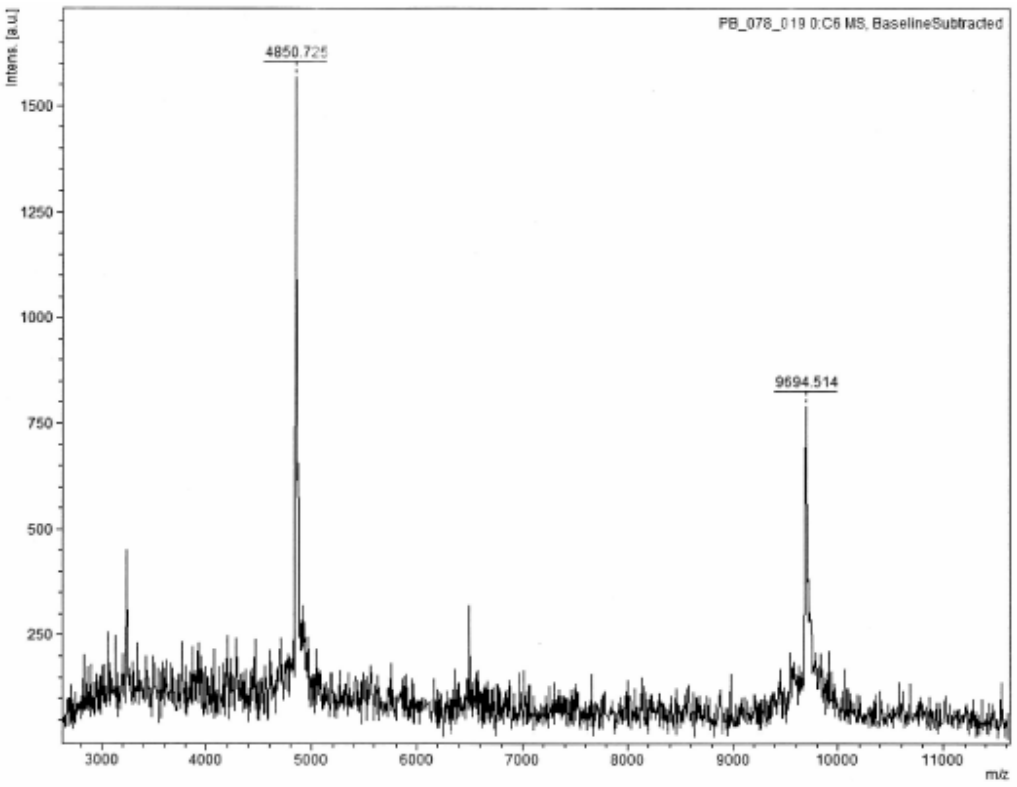
Scheme S195: HPLC-chromatogram of purified **DNA18**.



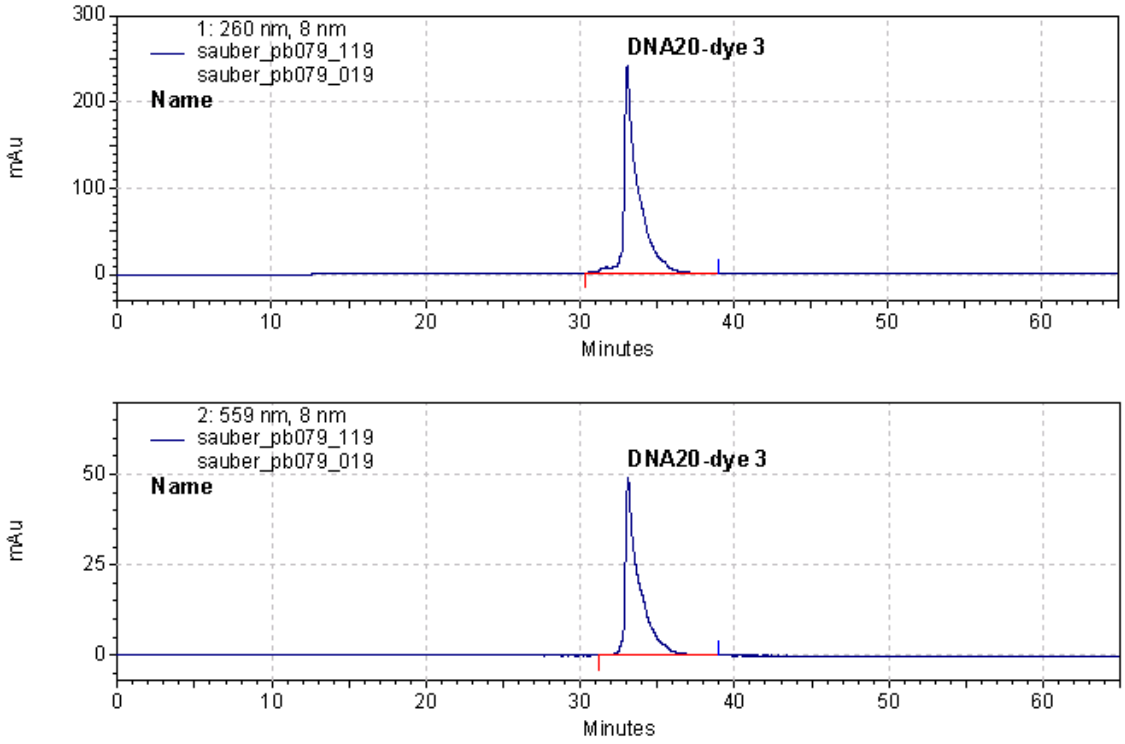
Scheme S196: MALDI spectra of **DNA18**, calculated: 9547.7 g/mol, found: 9548.9 g/mol.



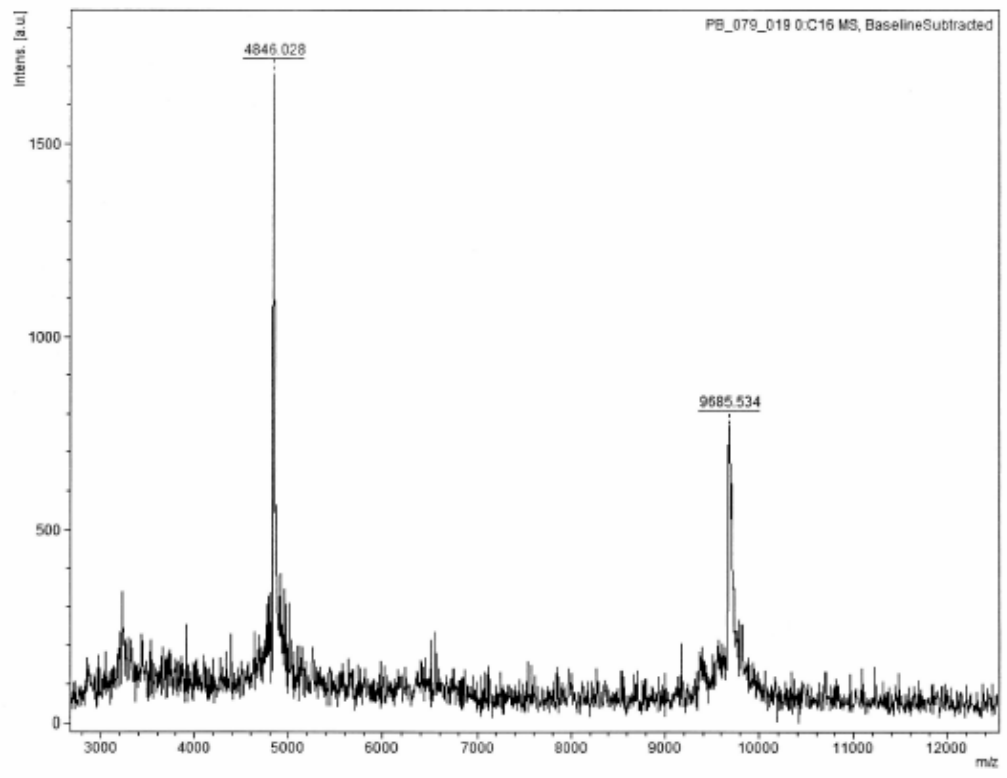
Scheme S197: HPLC-chromatogram of purified **DNA19**.



Scheme S198: MALDI spectra of **DNA19**, calculated: 9693.7 g/mol, found: 9694.5 g/mol.



Scheme S199: HPLC-chromatogram of purified **DNA20**.



Scheme S200: MALDI spectra of **DNA20**, calculated: 9679.7 g/mol, found: 9685.5 g/mol.

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