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Metal-mediated DNA assembly using the ethynyl linked terpyridine ligand

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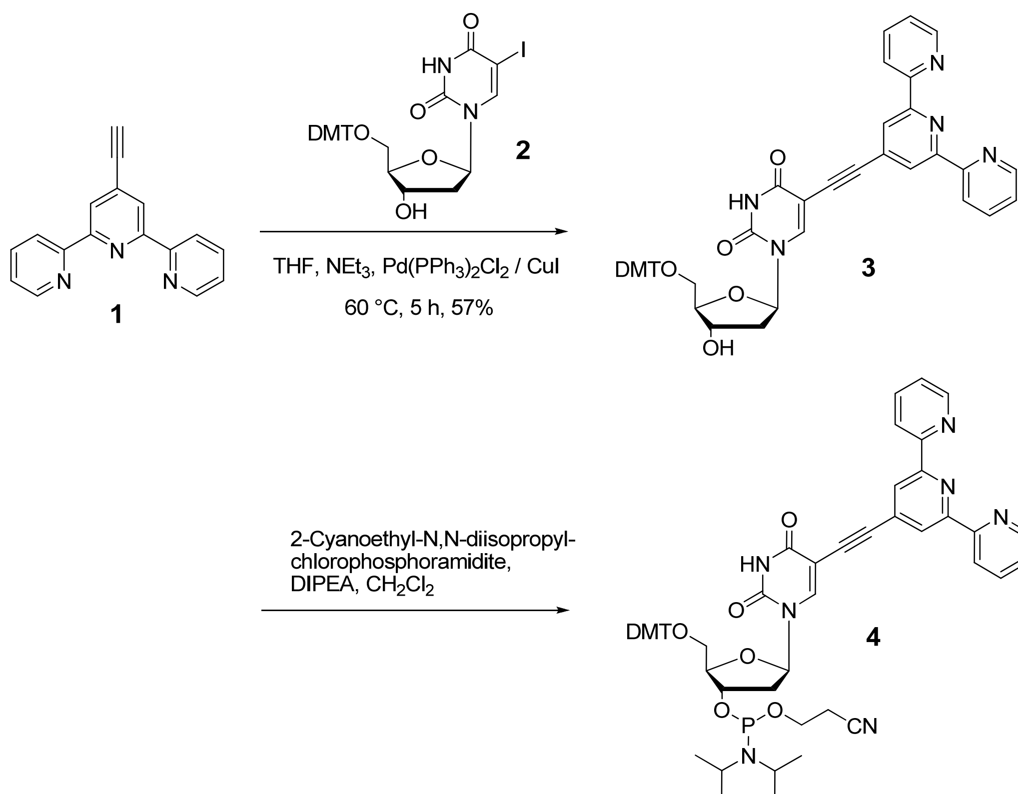
Materials and Methods

Chemicals and dry solvents were purchased from commercial suppliers and were used without further purification unless otherwise mentioned. TLC was performed on Fluka silica gel F254 coated aluminium foil. Flash chromatography was carried out with Silica Gel 60 from Aldrich (43 – 60 µm). Spectroscopic measurements were recorded in Na-Pi buffer solution (10 mM, pH 7) using quartz glass cuvettes (l = 10 mm). Mass spectra were measured in the central analytical facility of the institute. NMR spectra were recorded on a Bruker Advance 300 or Advance 600 spectrometer at 300 K in deuterated solvents. Chemical shifts are given in ppm relative to TMS. Absorption spectra and melting temperatures (2.5 µM DNA, 20 – 90 °C, 0.7 °C/min, step width 0.5 °C) were recorded on a Varian Carry 100 spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a step width of 1 nm an integration time of 0.2 s. All spectra were recorded with an excitation and emission bypass of 5 nm and are corrected for Raman emission of the buffer solution. The phosphoramidite of terpy-dU (**4**) was synthesized according to Scheme S1. 4´-Ethynyl-2,2´:6´,2´´-terpyridine (**1**) was synthesized according to literature reports.[1-3] DMT-protected 5-Iodo-deoxyuridine (**2**) was synthesized according to literature.[4]

Synthesis of the terpy-dU DNA building block



Scheme S1: Synthesis of the terpy-dU phosphoramidite building block

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(3) 5-[(2,2´:6´,2´´-Terpyridin-4´-yl)ethynyl]-5'-*O*-[(bis-(4-methoxyphenyl)(phenyl) methoxy]-2'-deoxyuridine

2 (460 mg, 0.70 mmol) was dissolved in 10 mL dry THF under Argon-Atmosphere.**1** (150 mg, 0.58 mmol), $Pd(PPh_3)_2Cl_2$ (40 mg, 0.057 mmol), CuI (11 mg, 0.058mmol) and abs. NEt_3 (5mL) were added and the reaction mixture was purged with Argon for 15 min. The mixture was heated to 60 °C for 5 h. The solvent was removed under reduced pressure and the crude product was pre-purified by flash chromatography (CH_2Cl_2/MeOH 10:1 + 3% NEt_3). The pure product **3** was obtain after a second purification step by flash chromatography on reversed phase (MeOH) as a white solid (264 mg, 0.34 mmol, yield: 58%).

^{1}H NMR (CD_2Cl_2, 600 MHz) **δ** 8.61 (d, 2H, J = 3.8 Hz), 8.56 (d, 1H, J = 7.9 Hz), 8.26 (s, 2H), 8.13 (s, 1H), 7.85 (t, 2H, J = 7.6 Hz), 7.41 (d, 2H, J = 7.6 Hz), 7.32 (m, 6H), 7.21 (t, 2H, J = 7.6 Hz), 7.11 (t, 1H, J = 7.3 Hz), 6.75 (m, 4H), 6.33 (t, 1H, J = 5.8 Hz), 4.61 (m, 1H), 4.14 (m, 1H), 3.60 (2 x s, 2 x 3H), 3.41 (dd, 1H, J_1 = 10.5 Hz, J_2 = 3.8 Hz), 3.31 (dd, 1H, J_1 = 10.5 Hz), 2.59 (m, 1H), 2.46 (td, 1H, J_1 = J_2 = 6.6 Hz); **^{13}C NMR** (CD_2Cl_2, 150 MHz) **δ** 161.6, 159.0, 159.0, 155.8, 155.8, 149.7, 149.5, 145.0, 144.0, 137.2, 136.1, 135.7, 132.8, 130.3, 130.2, 128.4, 128.2, 127.3, 124.4, 123.0, 121.4, 113.6, 113.6, 99.6, 91.7, 87.2, 86.9, 86.4, 85.3, 72.2, 64.1, 55.4, 41.8; **HR-FAB-MS** m/z (calcd.) = 786.2928 [M-H$^+$]; m/z (found) = 786.2932 [M-H$^+$]

(4) 5-[(2,2´:6´,2´´-Terpyridin-4´-yl)ethynyl]- 3'-*O*-((2-Cyanoethoxy) (diisopropylamino)phosphinyl)-5'-*O*-[(bis-(4-methoxyphenyl)(phenyl)methoxy]-2'-deoxyuridine

3 (100 mg, 0.13 mmol) was dissolved in 3 mL dry CH_2Cl_2 under Argon-Atmosphere. Dry DIPEA (110 uL, 0.64 mmol) and 2-Cyanoethyl*N,N*-diisopropylchlorophosphoramidite (114 µL, 0.51 mmol) were added and the mixture was stirred at room temperature over night. The reaction mixture was washed with freshly prepared aq. $NaHCO_3$ solution und the organic layer was dried over Na_2SO_4. The solvent was removed in vacuum to yield **4** (125 mg, 0.13 mmol, yield: 99%) which was used for DNA synthesis without further purification.

^{31}P NMR (d_6-DMSO, 121MHz) **δ** 148.4, 148.7;

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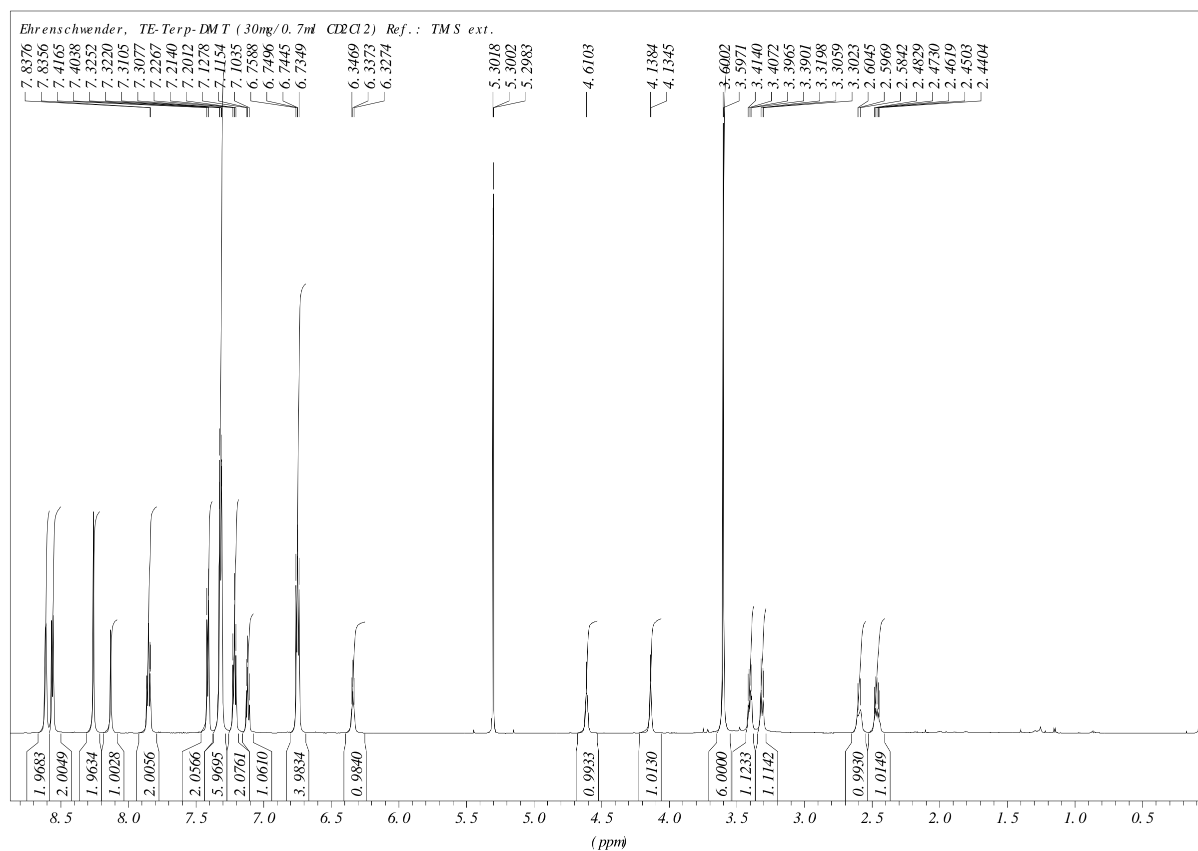
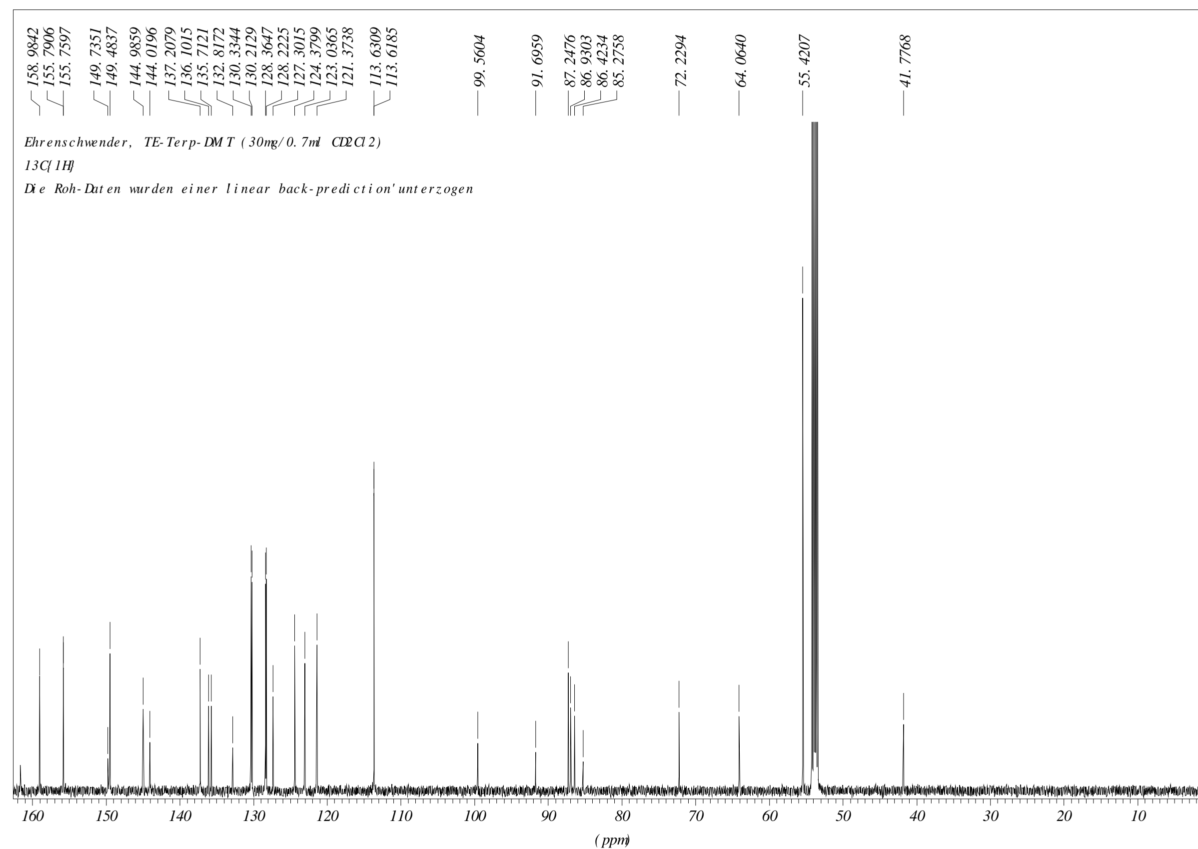
^{1}H-NMR of **3**:



^{13}C-NMR of **3**:



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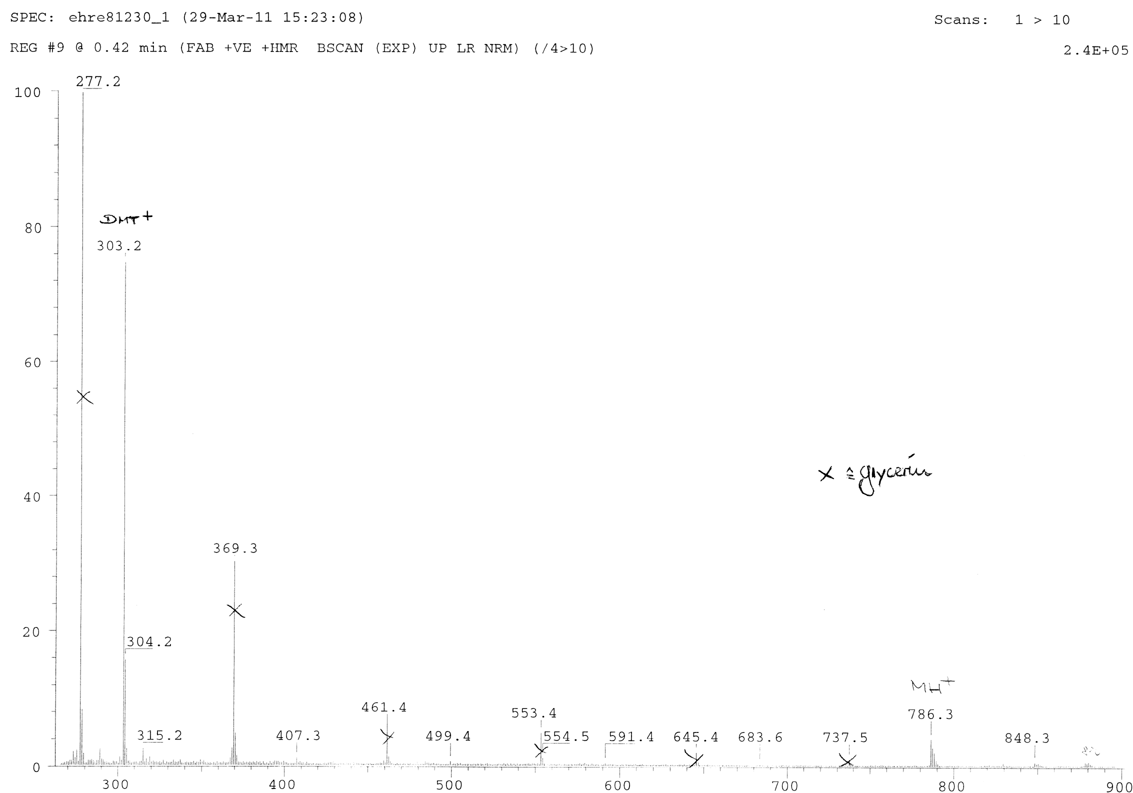
FAB-MS of **3:**



HR-FAB-MS of **3:**

UCLR USER LIST FILE (4x500): ehre2 SAVE

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2:	786.29384	0.00000	0.00000	0.00000
3:	786.29868	0.00000	0.00000	0.00000
4:	786.30046	0.00000	0.00000	0.00000
5:	786.29045	0.00000	0.00000	0.00000
6:	0.00000	0.00000	0.00000	0.00000
7:	0.00000	0.00000	0.00000	0.00000
8:	0.00000	0.00000	0.00000	0.00000
9:	0.00000	0.00000	0.00000	0.00000
10:	0.00000	0.00000	0.00000	0.00000
11:	0.00000	0.00000	0.00000	0.00000
12:	0.00000	0.00000	0.00000	0.00000
13:	0.00000	0.00000	0.00000	0.00000
14:	0.00000	0.00000	0.00000	0.00000
15:	0.00000	0.00000	0.00000	0.00000
16:	0.00000	0.00000	0.00000	0.00000
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19:	0.00000	0.00000	0.00000	0.00000
20:	0.00000	0.00000	0.00000	0.00000
LINK	NONE	NONE	NONE	NONE
SIZE	5.00000	0.00000	0.00000	0.00000
MEAN	786.29319	0.00000	0.00000	0.00000
SUM	3931.46597	0.00000	0.00000	0.00000
S.D.	0.00715	0.00000	0.00000	0.00000
MAX	786.30046	0.00000	0.00000	0.00000
MIN	786.28254	0.00000	0.00000	0.00000

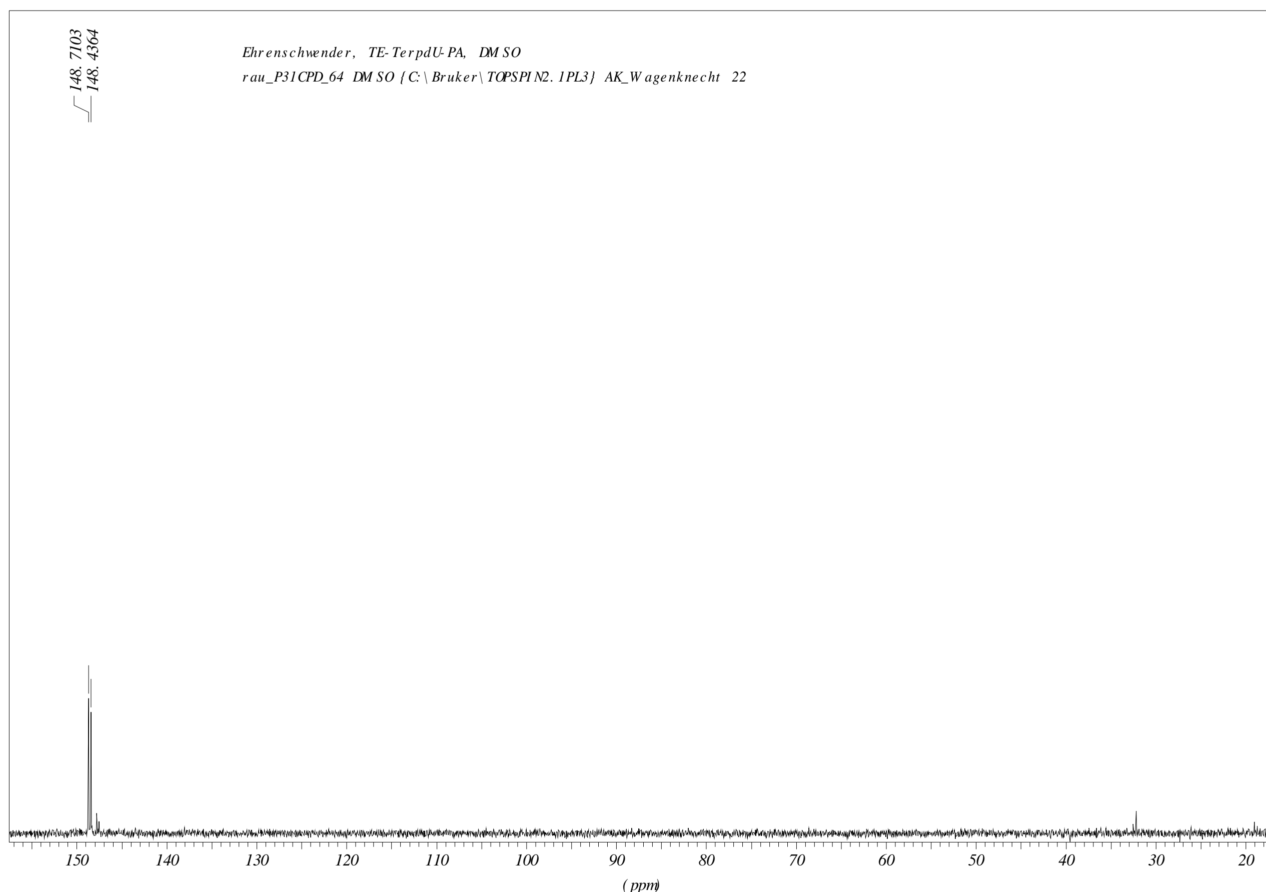
ULIST: _

```
Limt: (     0 )
    : ( 160 ) C100.H100.N10.O10
Peak:  400.00  mmu      R+D:  -0.5 > 50.0

Mass of { 786.2932 } = 786.293200000
Abs Abun.   .5680026412

Delta (ppm)    R+    Formula
-.54254       30.5   C47.H40.N5.O7
1.15845       35.5   C48.H36.N9.O3
1.16504       30.0   C49.H42.N2.O8
-1.1882       43.0   C61.H38.O
-2.2501       31.0   C45.H38.N8.O6
2.86603       35.0   C50.H38.N6.O4
-2.8958       43.5   C59.H36.N3
-3.9511       26.0   C44.H42.N4.O10
4.56702       40.0   C51.H34.N10
4.57361       34.5   C52.H40.N3.O5
-5.6587       26.5   C42.H40.N7.O9
6.27461       39.5   C53.H36.N7.O
6.28120       34.0   C54.H42.O6
-6.3043       39.0   C56.H38.N2.O3
-7.3663       27.0   C40.H38.N10.O8
7.98219       39.0   C55.H38.N4.O2
-8.0119       39.5   C54.H36.N5.O2
8.62783       26.5   C41.H40.N9.O8
 9.6898       38.5   C57.H40.N.O3
 -9.713       34.5   C53.H40.N.O6
 -9.720       40.0   C52.H34.N8.O
10.3354       26.0   C43.H42.N6.O9
-11.420       35.0   C51.H38.N4.O5
12.0364       31.0   C44.H38.N10.O5
12.0430       25.5   C45.H44.N3.O10
13.0983       43.0   C60.H38.N2
-13.121       30.0   C50.H42.O9
-13.128       35.5   C49.H36.N7.O4
13.7440       30.5   C46.H40.N7.O6
-14.829       30.5   C48.H40.N3.O8
-14.836       36.0   C47.H34.N10.O3
```

^{31}P NMR of **4:**



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Synthesis and Characterization of DNA1 – 6

The oligonucleotides were prepared on an Expetide 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1 µmol) with longer coupling times of 500 s and higher concentration of phosphoramidite (0.1 M). The chemicals for the DNA synthesis were purchased from ABI and Glen Reasearch. After preparation, the trityl-off oligonucleotides were cleaved of the resin and deprotected with conc. NH_4OH at 55°C for 10 h. The oligonucleotides were dried and purified by reversed phase HPLC using the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0 - 20% B over 40 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm on a Varian Carry 100 spectrometer. Duplexes of DNA1 – 6 were prepared by heating the modified oligonucleotides in presence of 1.2 equiv. of unmodified complementary strand to 90°C, followed by cooling to room temperature. If both strands were modified a 1:1 ratio was used for hybridization.

Table S1: MS analysis and extiction coefficients of the oligonucleotides **DNA1-6**

Oligonucleotide	Calculated mass [g/mol]	Observed mass [g/mol]	ε_{260} [mM^{-1} cm^{-1}]
DNA1	5390	1796.7 $[M-3H^+]^{3-}$	162.9
		1347.2 $[M-4H^+]^{4--}$	
DNA2	5457	1819.0 $[M-3H^+]^{3-}$	184.7
		1363.9 $[M-4H^+]^{4-}$	
DNA3	5440	1813.3 $[M-3H^+]^{3-}$	168.2
		1359.6 $[M-4H^+]^{4-}$	
DNA4	5409	1803.2 $[M-3H^+]^{3-}$	170.1
		1351.9 $[M-4H^+]^{4-}$	
DNA5	5405	1801.8 $[M-3H^+]^{3-}$	164.3
		1351.0 $[M-4H^+]^{4-}$	
DNA6	5450	1816.8 $[M-3H^+]^{3-}$	194.0
		1362.2 $[M-4H^+]^{4-}$	

Melting temperatures of DNA3 with different counter bases for terpy-dU

All melting temperatures were determined by the change of absorbance at 260nm using the following conditions: 2.5 µM DNA, 10 mM NaPi buffer, 250 mM NaCl; heating/cooling rate 0.7°C/min, data interval 0.5°C.

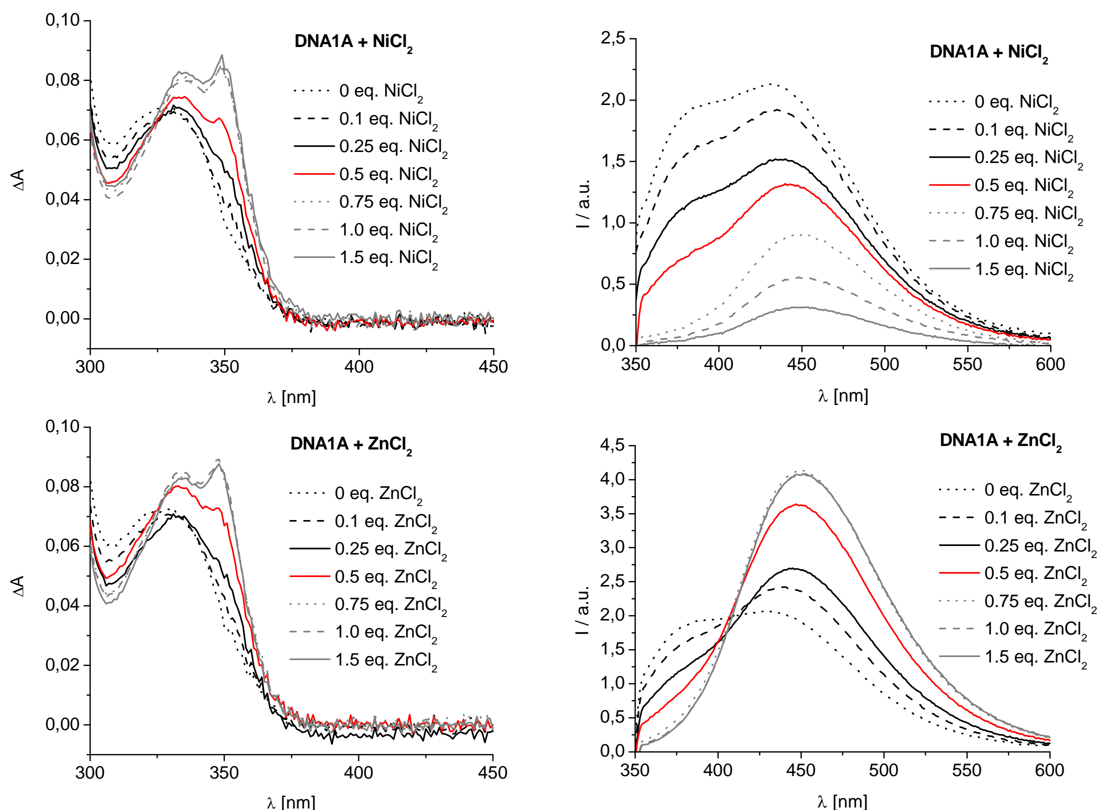
Table S2: Melting temperatures for DNA3 with different counter bases for terpy-dU; ΔTm values are calculated compared to the fully matched unmodified duplex with T instead of terpy-dU (T_m = 68.0°C)

Duplex	T_m [°C]	ΔT_m [°C]
DNA3A	63.9	- 4.1
DNA3T	63.2	- 4.8
DNA3G	60.3	- 7.7
DNA3C	60.8	- 7.2

Titration experiments with terpy-dU modified oligonucleotides

The oligonucleotides were hybridised in absence of metal-ions. For the titration experiments freshly prepared 200 µM $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $ZnCl_2$ solutions were used. To allow a good complexation we waited 90 min between the single titration steps. The emission spectra were recorded after excitation at 345 nm. The following concentrations were used for all experiments: 2.5 µM modified DNA, 3.0 µM unmodified counter strand, 10 mM NaPi buffer, 250 mM NaCl.

Titration experiments with **DNA1A** and **DNA5A**



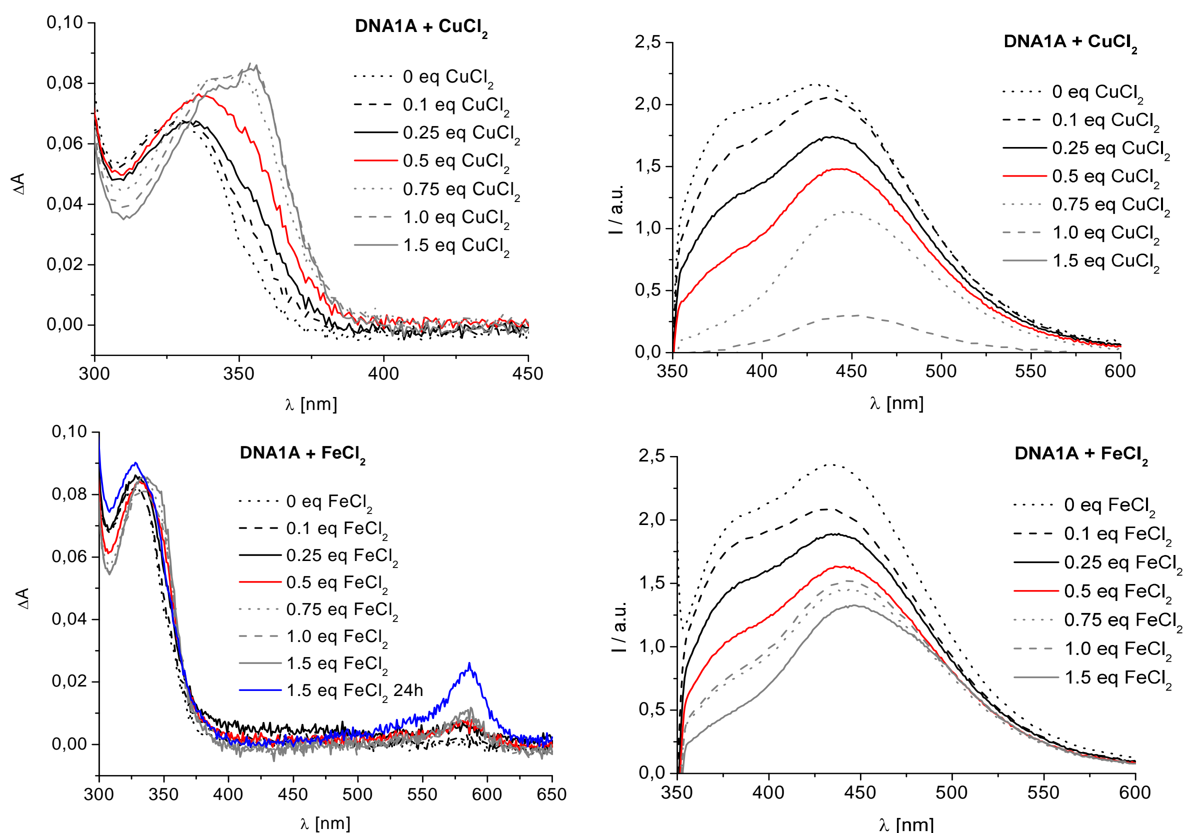
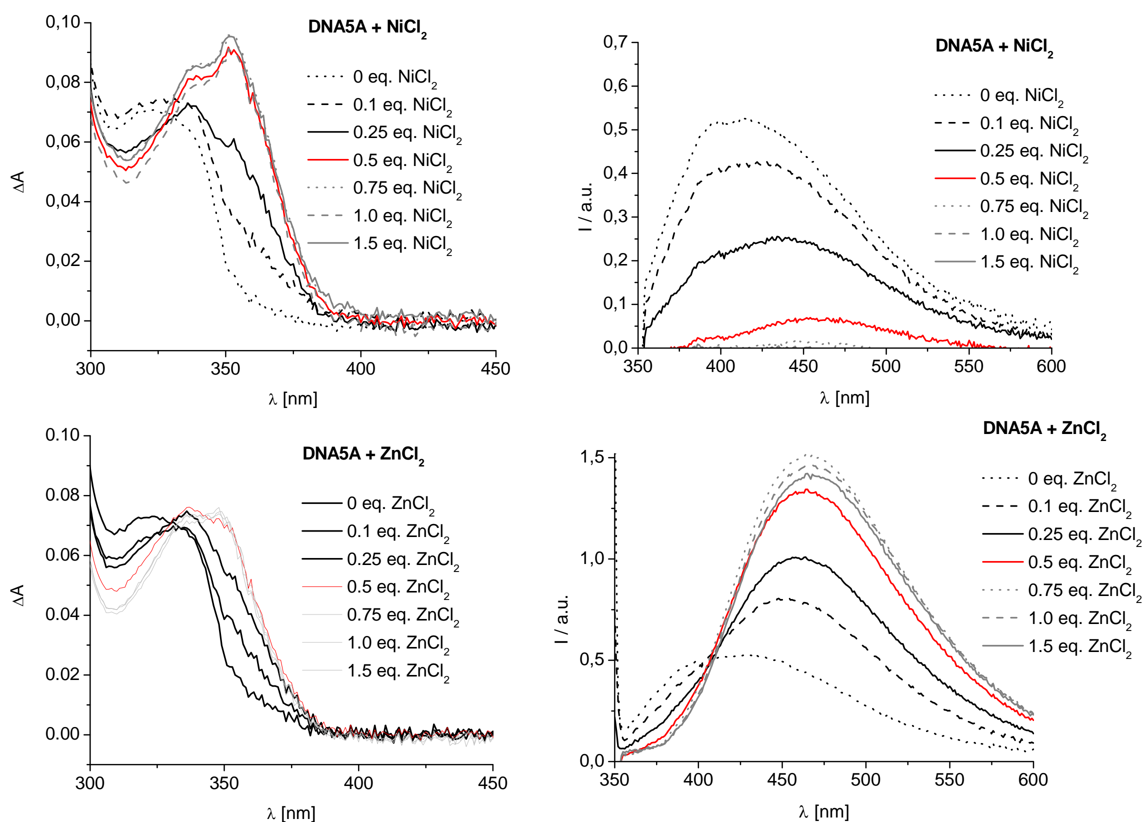


Fig. S1: Changes in the absorption and emission spectra of **DNA1A** upon addition of $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_2$ (λ_{ex} = 345 nm)



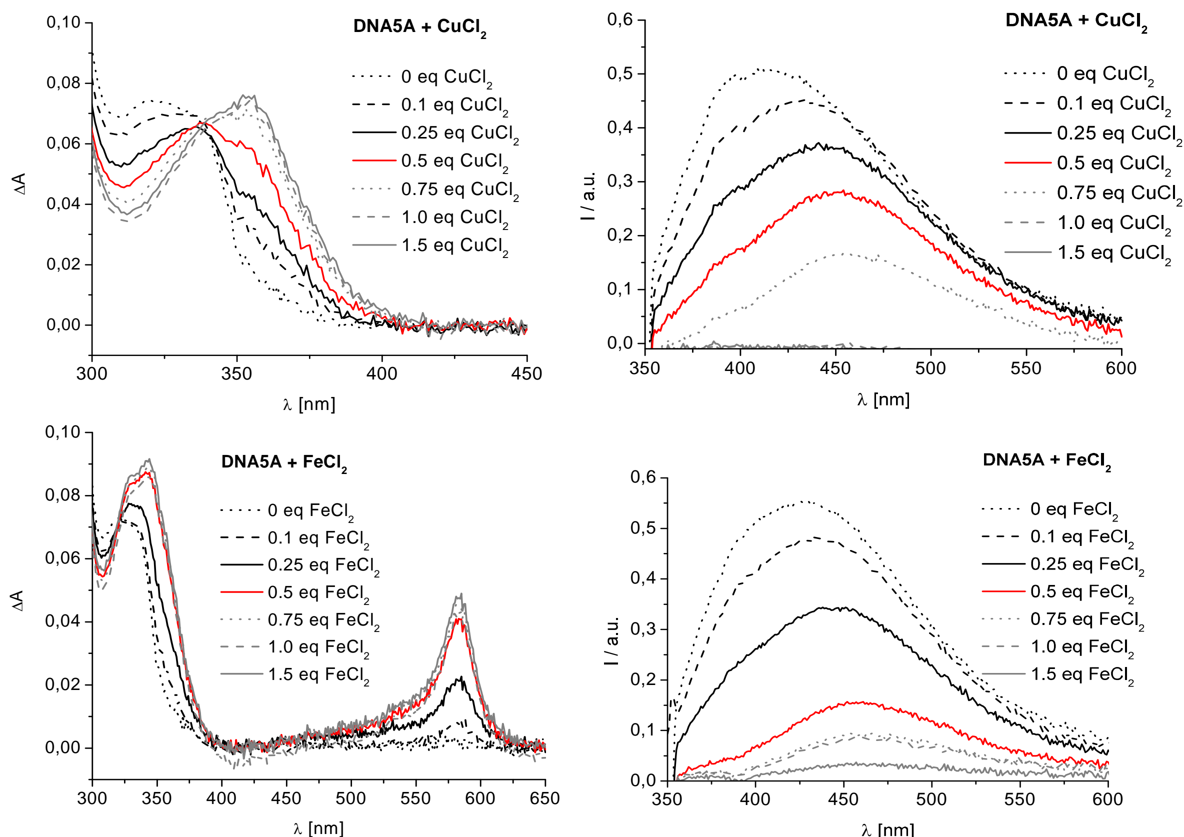
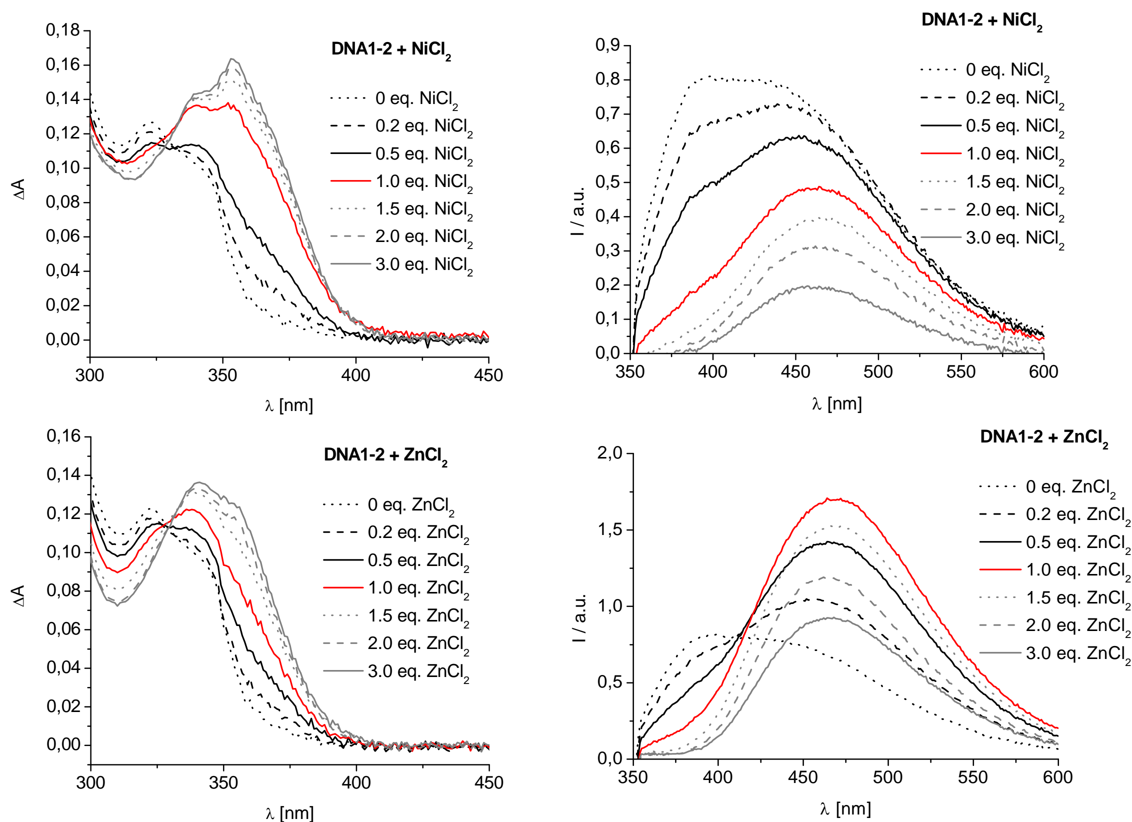


Fig. S2: Changes in the absorption and emission spectra of **DNA5A** upon addition of $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_2$ ($\lambda_{ex} = 345$ nm)

Titration experiments with **DNA1-2** and **DNA5-6**



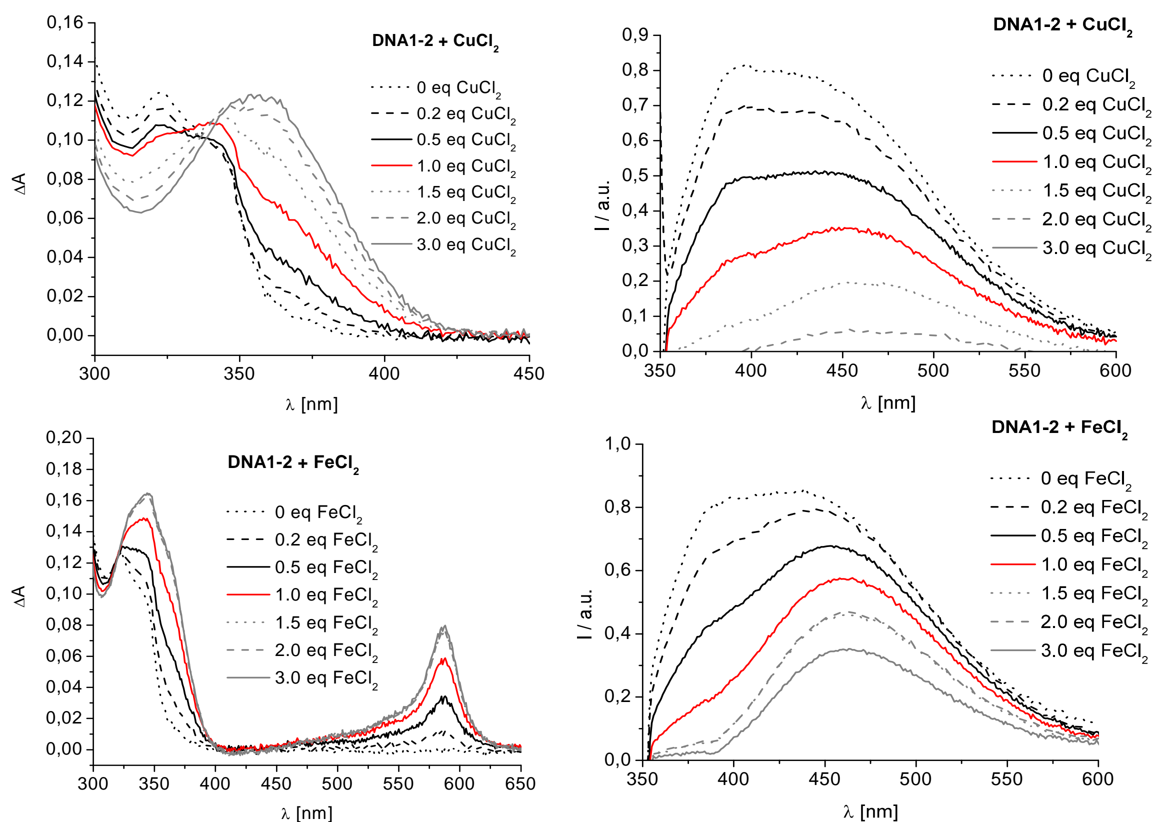
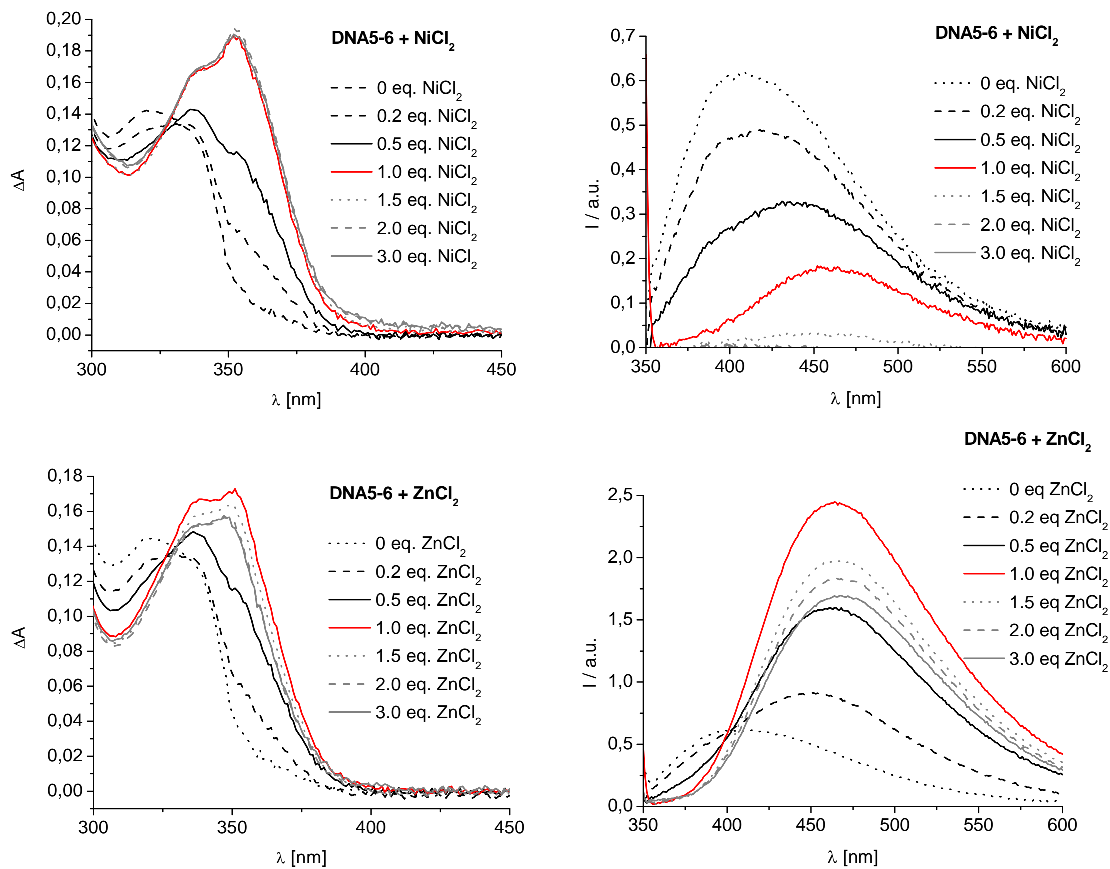


Fig. S3: Changes in the absorption and emission spectra of **DNA1-2** upon addition of $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_2$ (λ_{ex} = 345 nm)



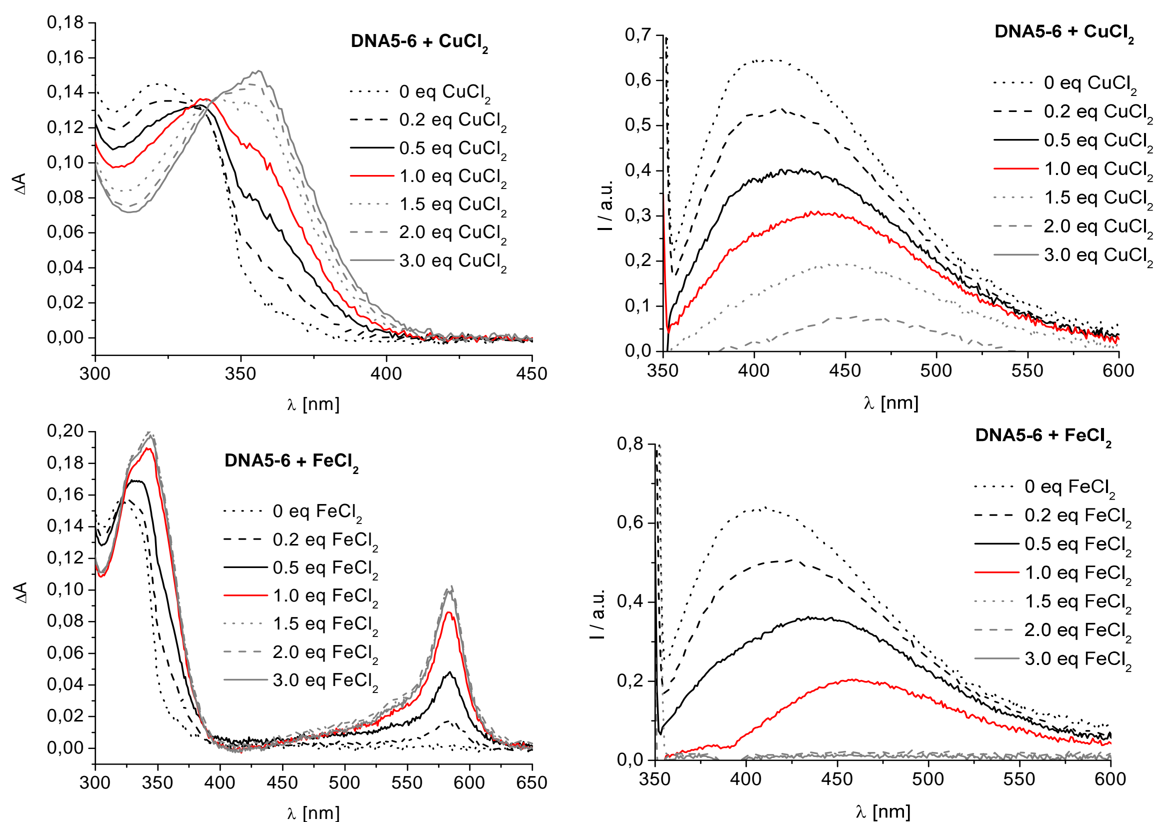


Fig. S 4: Changes in the absorption and emission spectra of **DNA5-6** upon addition of $NiCl_2$, $ZnCl_2$, $CuCl_2$ and $FeCl_2$ (λ_{ex} = 345 nm)

SB-PAGE Experiments

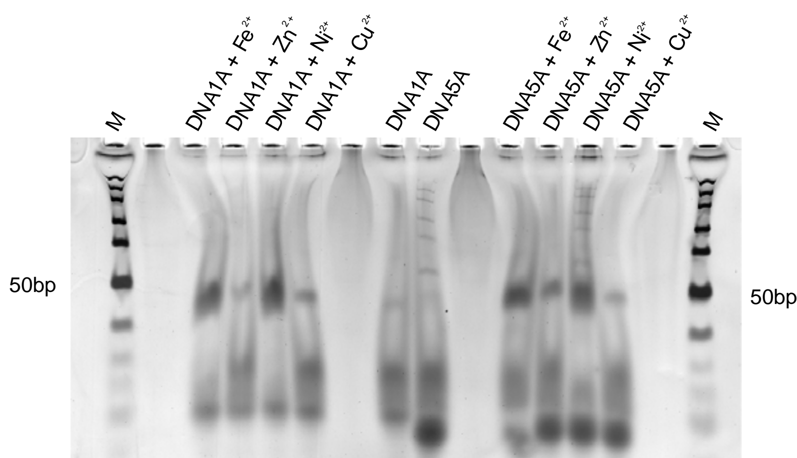


Fig. S 5: Top: Fluorescence quenching (Fq) for **DNA1A** (left) and **DNA5A** (right) upon addition of metal ions; bottom: non-denaturing gel electrophoresis (8 % SB-PAGE) of **DNA1A** and **DNA5A** in absence (middle lanes) and presence of metal ions after silver staining.

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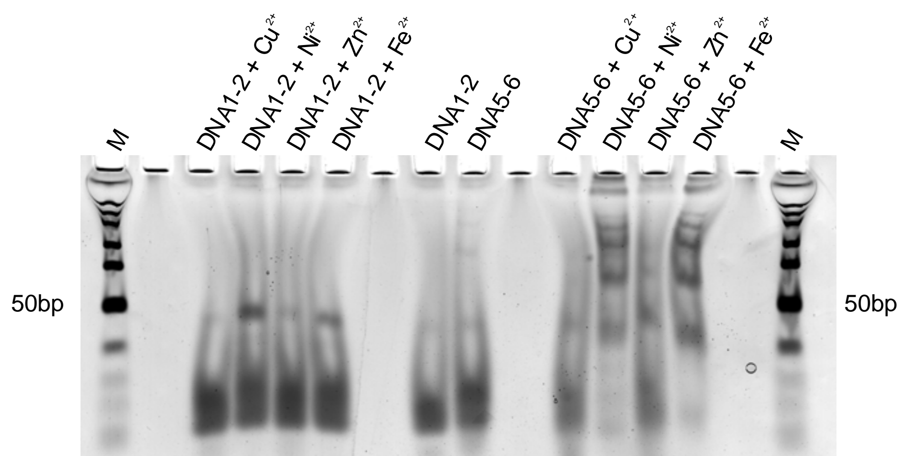

Fig. S 5: Top: Fluorescence quenching (Fq) for **DNA1-2** (left) and **DNA5-6** (right) upon addition of metal ions; bottom: non-denaturing gel electrophoresis (8 % SB-PAGE) of **DNA1-2** and **DNA5-6** in absence (middle lanes) and in presence of metal ions after silver staining.

References

1. E. C. Constable, M. D. Ward, *J. Chem. Soc., Dalton Trans.* **1990**, 1405-1409.
2. K. T. Potts, D. Konwar, *J. Org. Chem.* **1991,** *56*, 4815-4816.
3. V. Grosshenny, F. M. Romero, R. Ziessel, *J. Org. Chem.* **1997,** *62*, 1491-1500.
4. R. H. E. Hudson, A. Ghorbani-Choghamarani, *Synlett* **2007**, 870-873.