Electronic Supplementary Material (ESI) for Organic .

Supporting Information

The base discriminating potential of pyrrolidinyl PNA demonstrated by magnetic Fe_xO_y particles

Claudia Stubinitzky,[a] Tirayut Vilaivan*[b] and Hans-Achim Wagenknecht*[a]

Institute of Organic Chemistry, Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany. Fax: +49-721-608-44825; Tel: +49-721-608-47486; E-mail: Wagenknecht@kit.edu

Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand, Fax: 02-2187598 Tel: 02-2187627 ext 101 E-mail: vtirayut@chula.ac.th

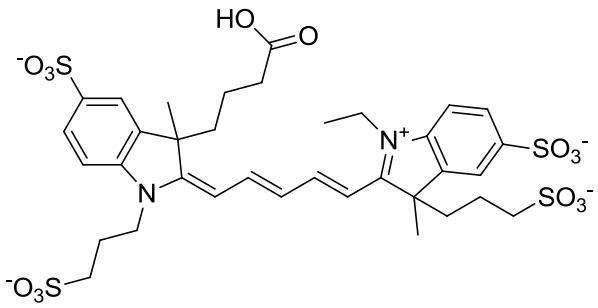
Contents **Page**

1. Materials and Methods

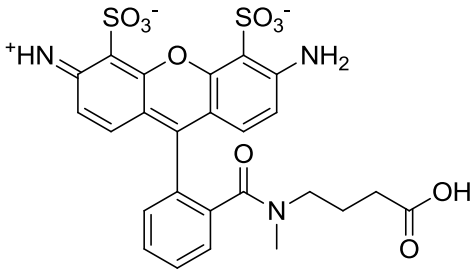
DNA0-DNA5 have been purchased from metabion international AG, DNA6-9 from biomers.net GmbH and pierce streptavidin magnetic beads from Thermo Fisher Scientific (Streptavidin monolayer covalently coupled to magnetic bead surface, bead concentration: 10mg/ml, binding capacitiy: ~ 3500 pmol biotinylated fluorescein/mg of beads).

Structures of Atto488 and DY649

DY649



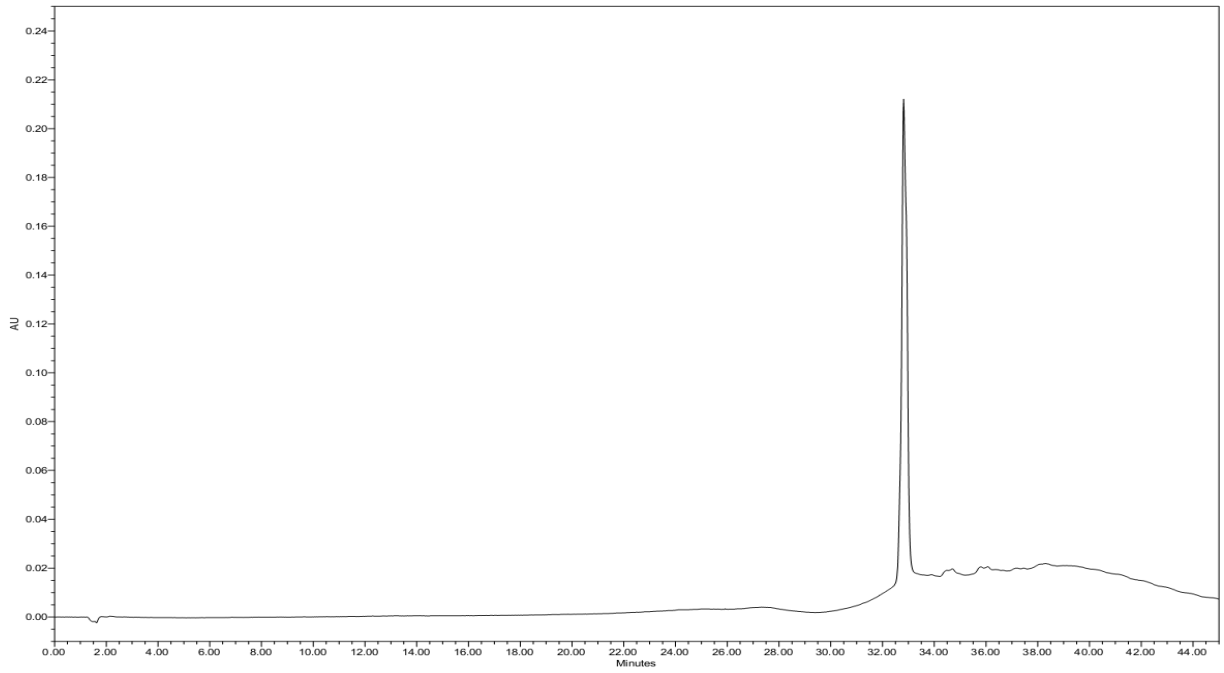
Atto488



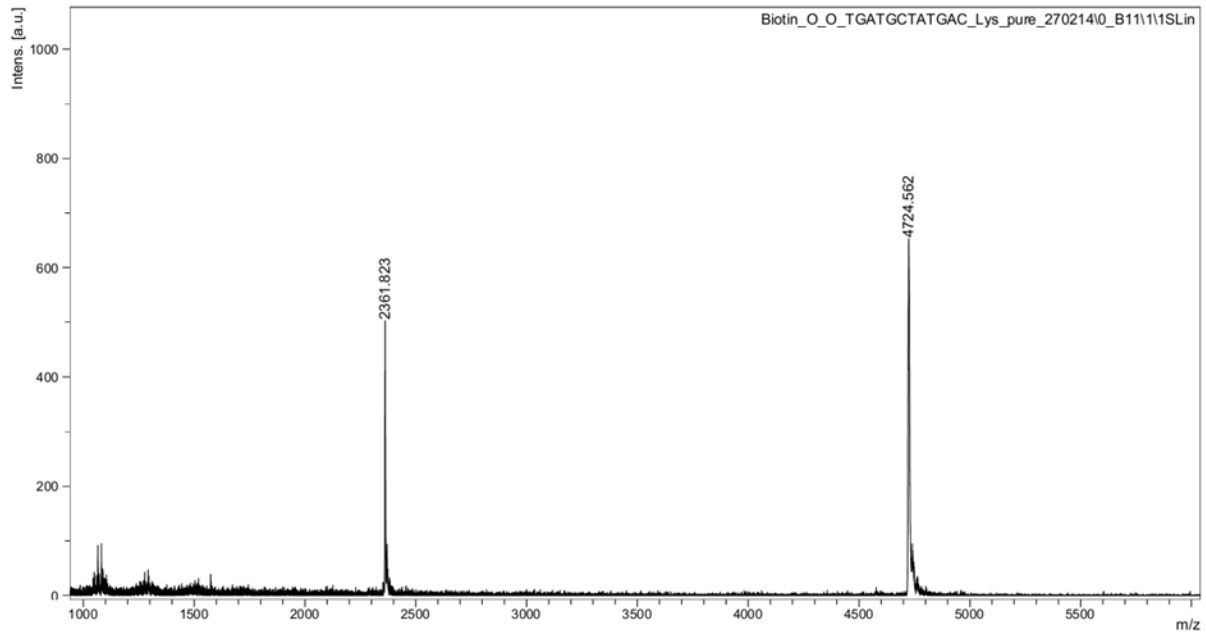
Synthesis of PNA1-PNA5

The PNAs were synthesized manually by Fmoc-solid phase peptide synthesis on TentaGel S RAM resin (0.24 mmol/g substitution, 1.5 µmol for PNA2 and 1 µmol for PNA1,3-5) preloaded with Lys(Boc) using the standard protocol.[1,2] Acpc PNA was modified at the N-terminus with biotin via two aminoethoxyethyoxyacetyl linkers. The nucleobase protecting groups (Bz and Ibu) were removed by NH_4OH:dioxane (1:1) treatment at 60 ºC overnight. Cleavage of the PNA oligomers from the resin was achieved by treatment with trifluoroacetic acid (TFA). After precipitation with diethyl ether, the PNAs were purified by reverse phase HPLC using the following conditions: A = 0.1% TFA in water; B = 0.1% TFA in MeOH; gradient = 0-80% B over 70 min. The collected fractions were lyophilized and quantified by their absorbance at 260 nm. Identity was verified by MS (MALDI) in the linear negative mode (matrix: saturated CCA solution with H_2O:acetonitrile (1:1) + 0.1 % TFA) and purity was verified by analytical reverse phase HPLC.

PNA1



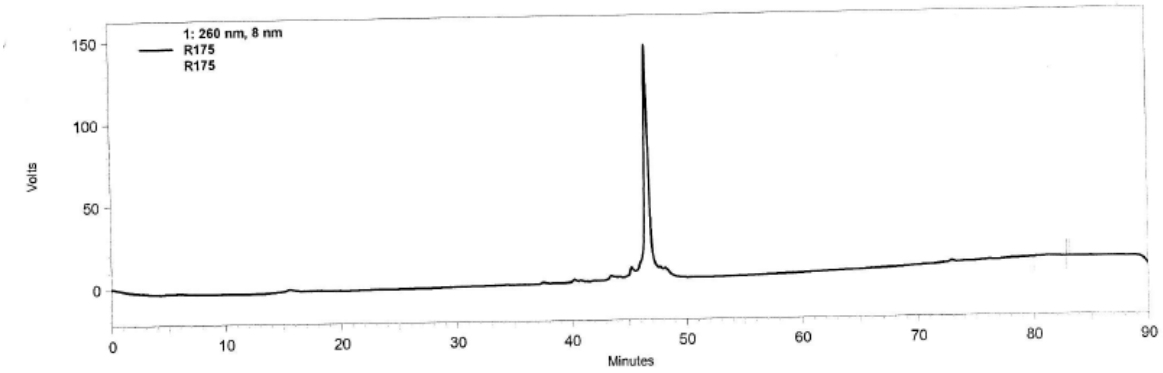
(HPLC conditions: C18 column 4.6×50 mm 3 µ, gradient H_2O:MeOH containing 0.1% TFA 90:10 for 5 min then linear gradient to 10:90 over 30 min, 260 nm)

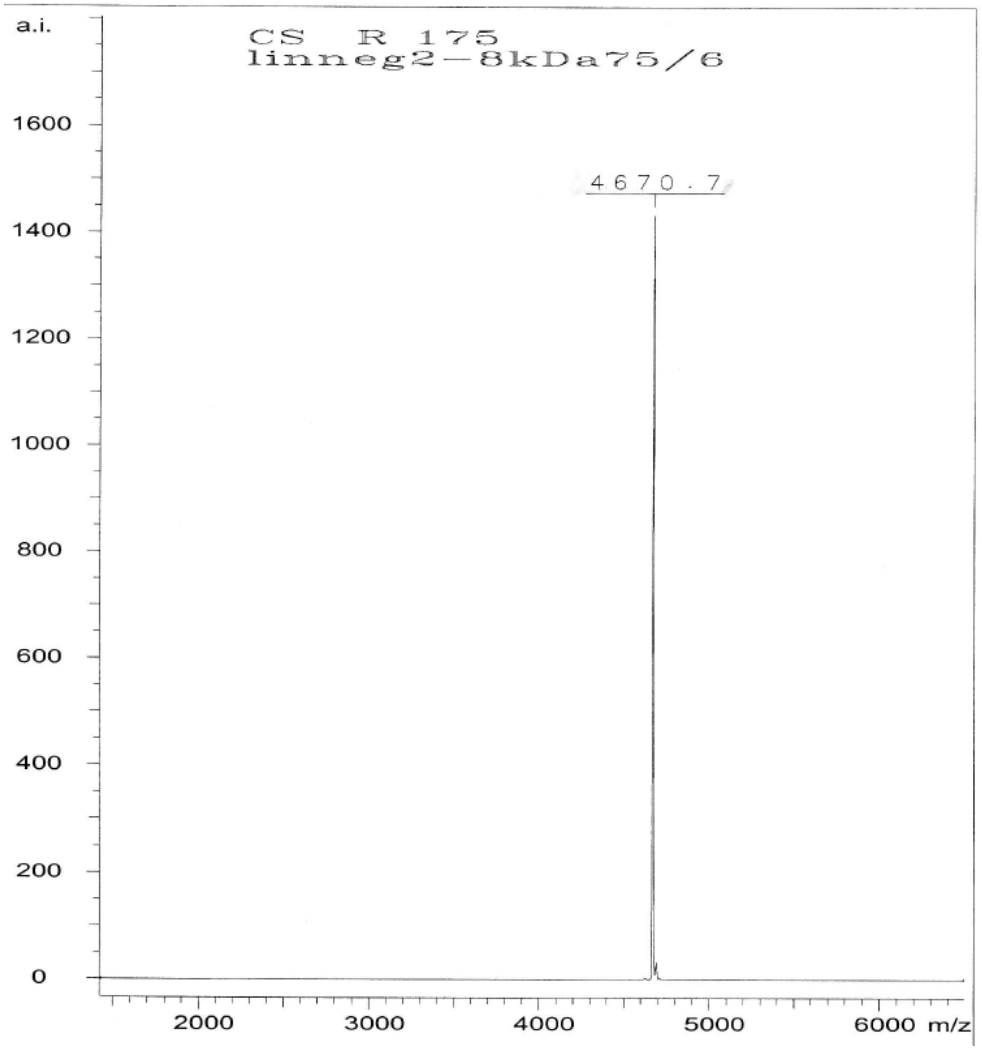


$M{\cdot}H^+$ (calculated) = 4723.1 g/mol $M{\cdot}H^+$ (found) = 4724.6 g/mol

$[M{\cdot}2H]^{2+}$ (calculated) = 2362.1 g/mol $[M{\cdot}2H]^{2+}$ (found) = 2361.8 g/mol

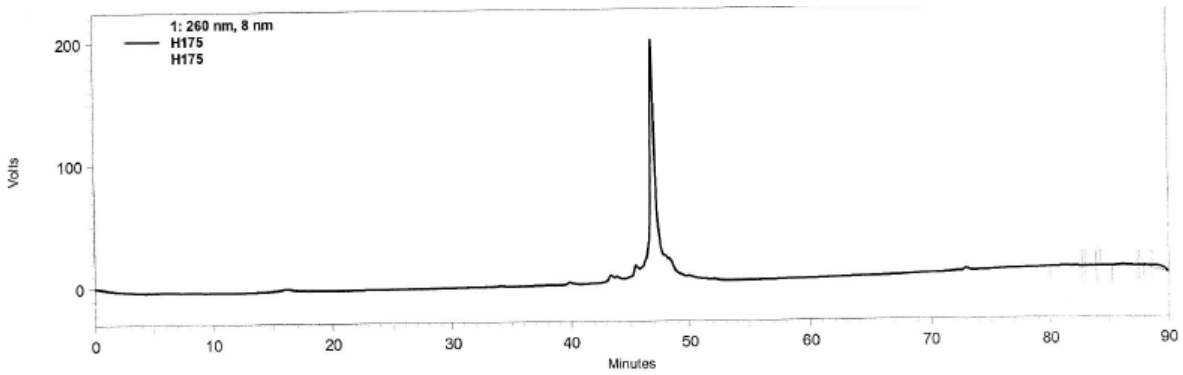
PNA2

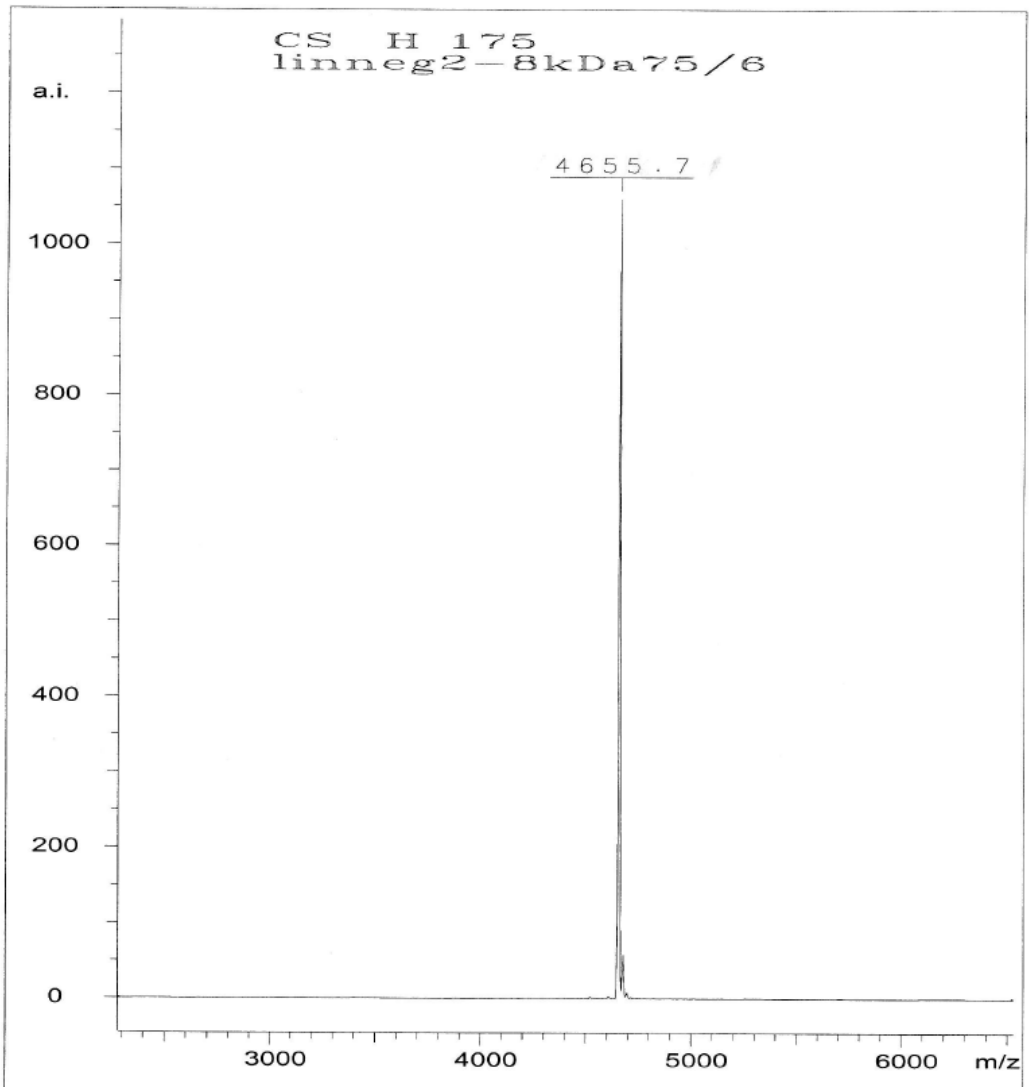




$M{\cdot}H^+$ (calculated) = 4670.1 g/mol $M{\cdot}H^+$ (found) = 4670.7 g/mol

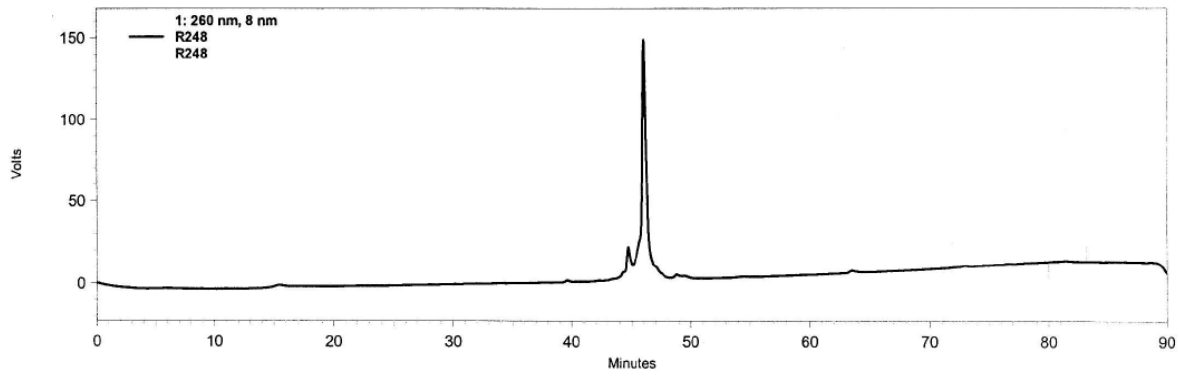
PNA3

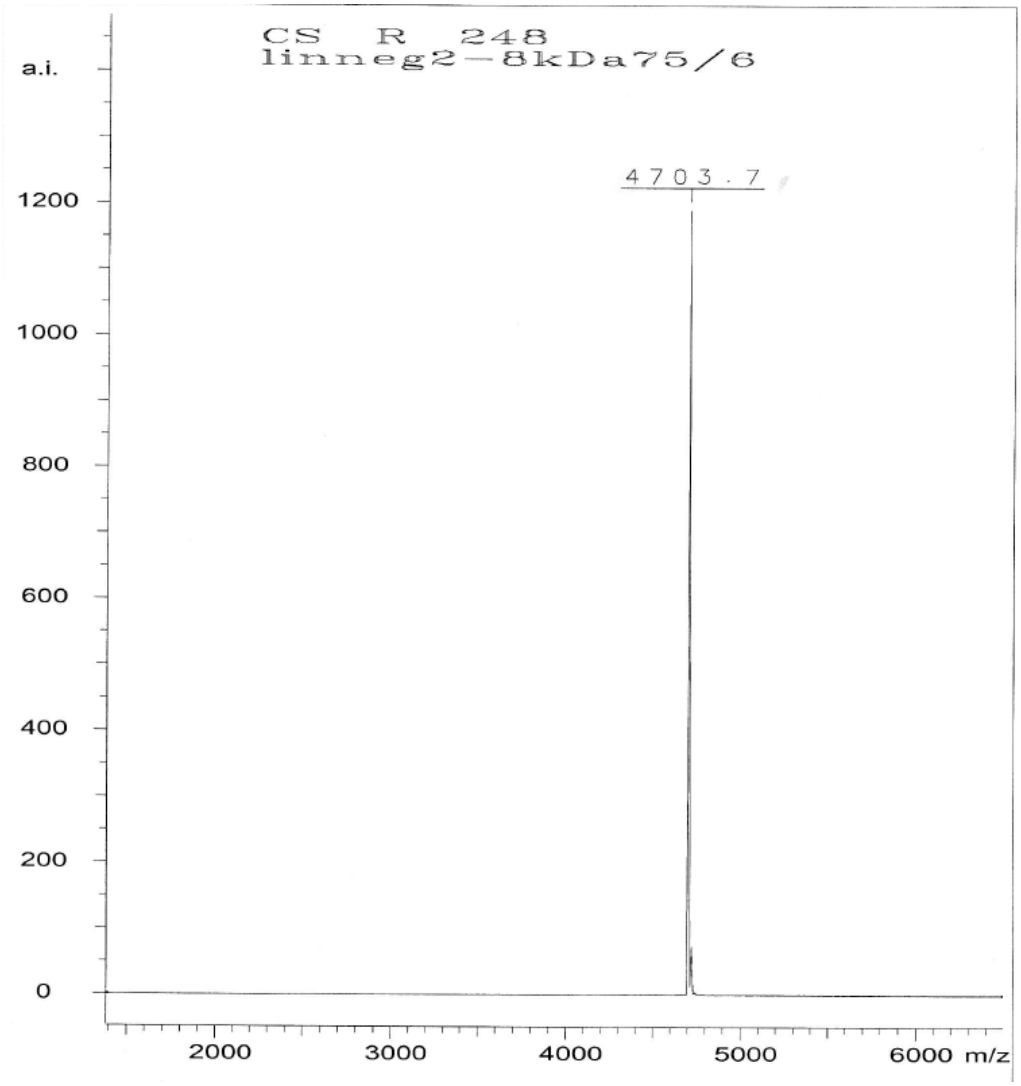




$M{\cdot}H^+$ (calculated) = 4654.1 g/mol $M{\cdot}H^+$ (found) = 4655.7 g/mol

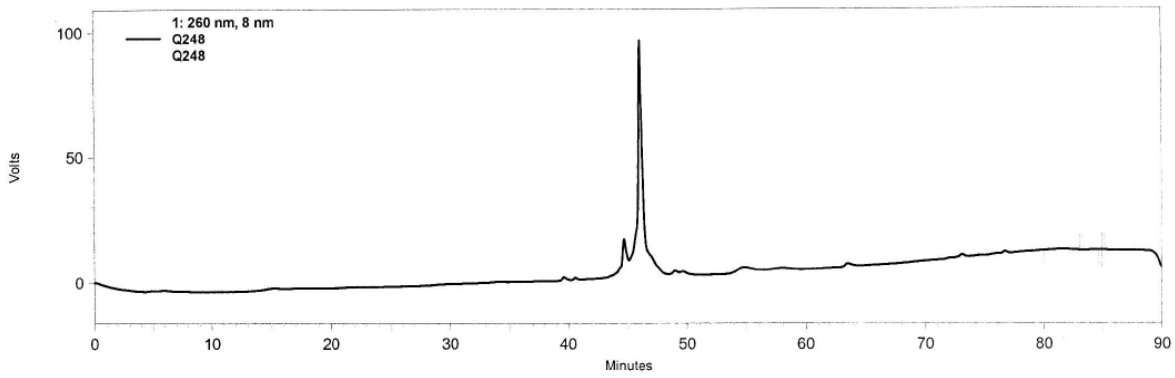
PNA4

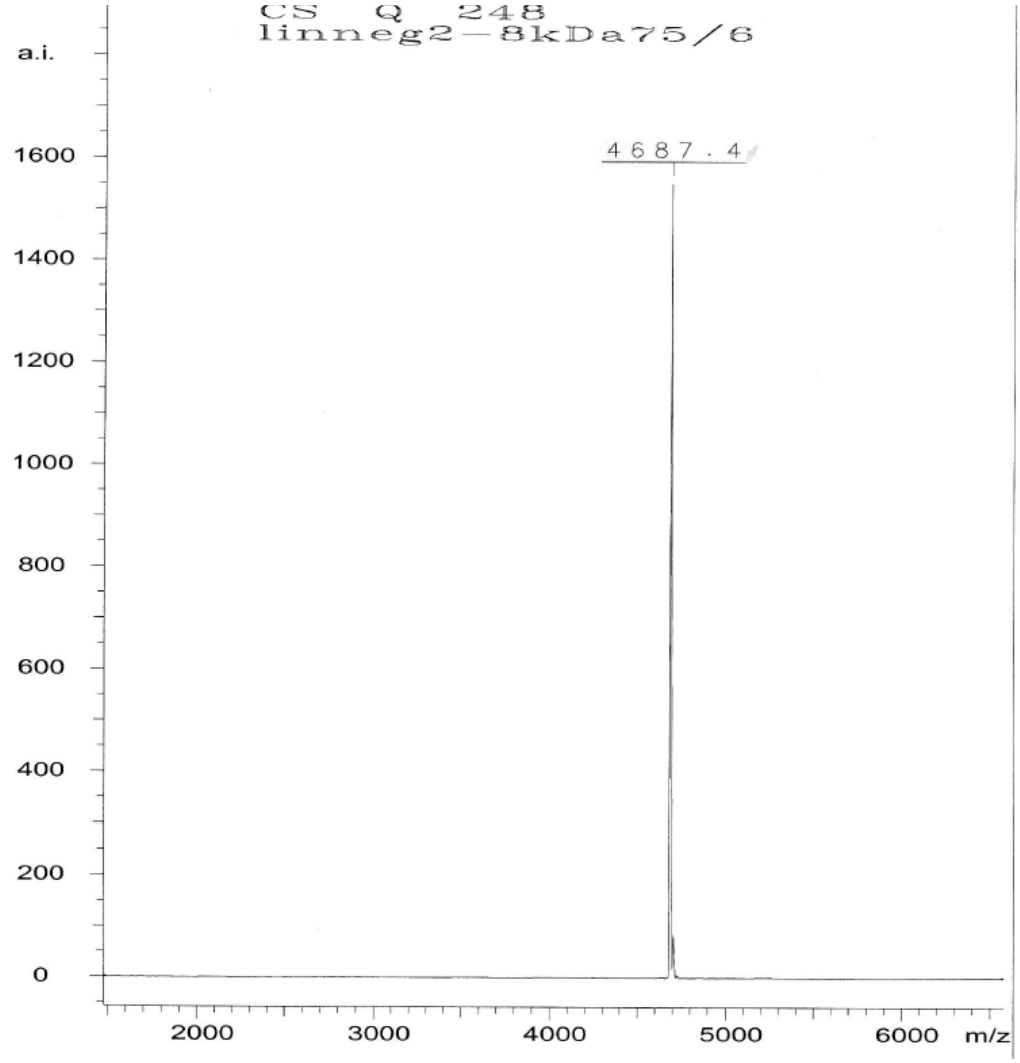




$M{\cdot}H^+$ (calculated) = 4703.1 g/mol $M{\cdot}H^+$ (found) = 4703.7 g/mol

PNA5





$M{\cdot}H^+$ (calculated) = 4687.1 g/mol $M{\cdot}H^+$ (found) = 4687.4 g/mol

2. PNA/DNA sequences and melting temperatures

Table S1: Sequences concerning R175H mutation

PNA2:	Biotin-O-O-AGGCGCTGCCCC-LysNH_2
PNA3:	Biotin-O-O-AGGCACTGCCCC-LysNH_2
DNA10	5´-TGCGGGGCAGCGCCT-3´
DNA11	5´-TGCGGGGCAGTGCCT-3´
DNA12	5´-AGGCGCTGCCCC-3´
DNA13	5´-AGGCACTGCCCC-3´

Table S2: Sequences concerning R248Q mutation

PNA4	Biotin-O-O-AACCGGAGGCCC-LysNH_2
PNA5	Biotin-O-O-AACCAGAGGCCC-LysNH_2
DNA14	5´-TGCGGGCCTCCGGTT-3´
DNA15	5´-TGCGGGCCTCTGGTT-3´
DNA16	5´-AACCGGAGGCCC-3´
DNA17	5´-AACCAGAGGCCC-3´

Melting temperatures

T_m were recorded with a Varian Cary 100 spectrometer using 2.5µM PNA-DNA / DNA-DNA solutions in 10 mM NaP_i buffer (pH =7) and 250 mM NaCl: λ = 260 nm, 10-90 °C, interval: 0.7 °C/min.

Table S3: T_m of **PNA2-3**, **DNA12-13** with **DNA10-11**

PNA	DNA	T_m [°C]	
PNA2	**DNA10**	69.2	ΔT_m = 16.0 °C
PNA2	**DNA11**	53.2	
PNA3	**DNA10**	36.3	ΔT_m = 33.5 °C
PNA3	**DNA11**	69.8	
DNA12	**DNA10**	66.3	ΔT_m = 10.6 °C
DNA12	**DNA11**	55.8	
DNA13	**DNA10**	43.1	ΔT_m = 19.2 °C
DNA13	**DNA11**	62.3	

Table S4: T_m of **PNA2-3** with **DNA2-3/DNA6-7**

PNA	DNA	T_m [°C]	
PNA2	**DNA2**	65.1	ΔT_m = 15.5 °C
PNA2	**DNA3**	49.6	
PNA2	**DNA6**	67.4	ΔT_m = 11.4 °C
PNA2	**DNA7**	56.0	
PNA3	**DNA2**	29.3	ΔT_m = 34.8 °C
PNA3	**DNA3**	64.1	
PNA3	**DNA6**	35.9	ΔT_m = 38.0 °C
PNA3	**DNA7**	73.9	

Table S5: T_m of **PNA4-5**, **DNA16-17** with **DNA14-15**

PNA	DNA	T_m [°C]	
PNA4	**DNA14**	70.0	ΔT_m = 13.4 °C
PNA4	**DNA15**	56.6	
PNA5	**DNA14**	47.2	ΔT_m = 21.1 °C
PNA5	**DNA15**	68.3	
DNA16	**DNA14**	63.9	ΔT_m = 10.4 °C
DNA16	**DNA15**	53.5	
DNA17	**DNA14**	45.9	ΔT_m = 15.2 °C
DNA17	**DNA15**	61.1	

Table S6: T_m of **PNA4-5** with **DNA4-5/DNA8-9**

PNA	DNA	T_m [°C]	
PNA4	**DNA4**	70.7	ΔT_m = 15.1 °C
PNA4	**DNA5**	55.6	
PNA4	**DNA8**	70.5	ΔT_m = 13.4 °C
PNA4	**DNA9**	57.1	
PNA5	**DNA4**	43.3	ΔT_m = 25.5 °C
PNA5	**DNA5**	68.8	
PNA5	**DNA8**	46.3	ΔT_m = 23.1 °C
PNA5	**DNA9**	69.4	

3. Spectroscopic analysis of capture experiments

3.1. General remarks

All absorption spectra were recorded with a Varian Cary 100 spectrometer using 2.5 µM solutions (V = 1 mL) in 10 mM NaP_i buffer (pH = 7) and 250 mM NaCl at 20°C, except otherwise mentioned. Oligonucleotide solutions were added to particles, after shaking they were kept for a certain time (specified in spectra). After capturing the Fe_xO_y particles with a magnet, the absorbance of the supernatant was measured spectrophotometrically.

3.2. Immobilisation of biotinylated oligonucleotides and biotinylated PNA

150 µL of magnetic streptavidin coated Fe_xO_y particles (marked with "P" in the spectra) were added into an Eppendorf tube and washed three times with milliQ water (3×1 mL). Afterwards the biotinylated oligonucleotide (DNA0 or biotinylated PNA) was added for immobilization for 15min.

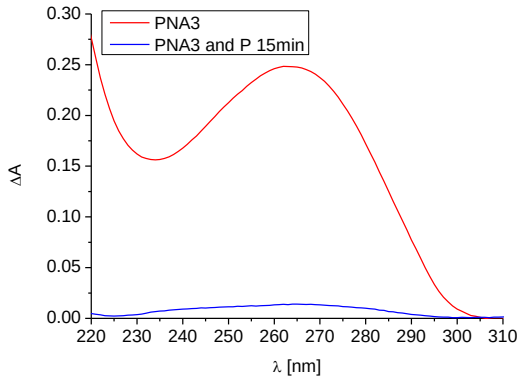


Figure S1: Immobilisation of **PNA3** on Fe_xO_y particles.

3.3. UV/Vis absorption spectroscopy of capture experiments

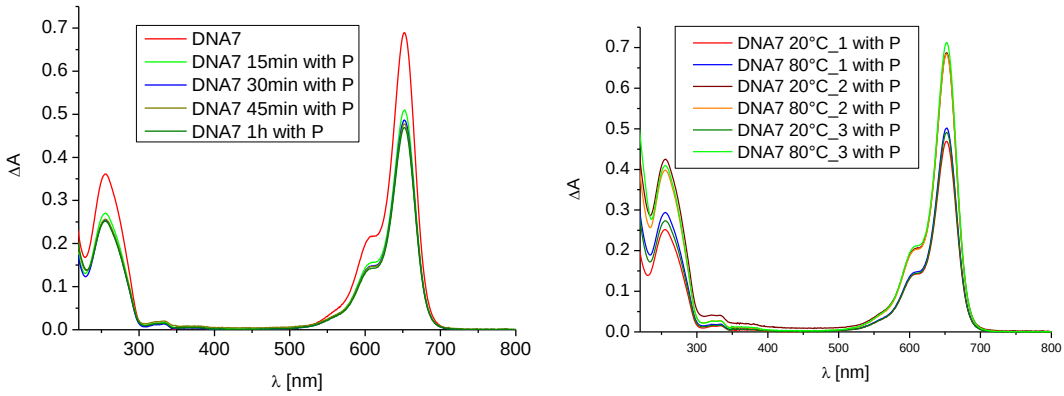


Figure S2: Measurements of **DNA7** with **PNA3** bearing particles.

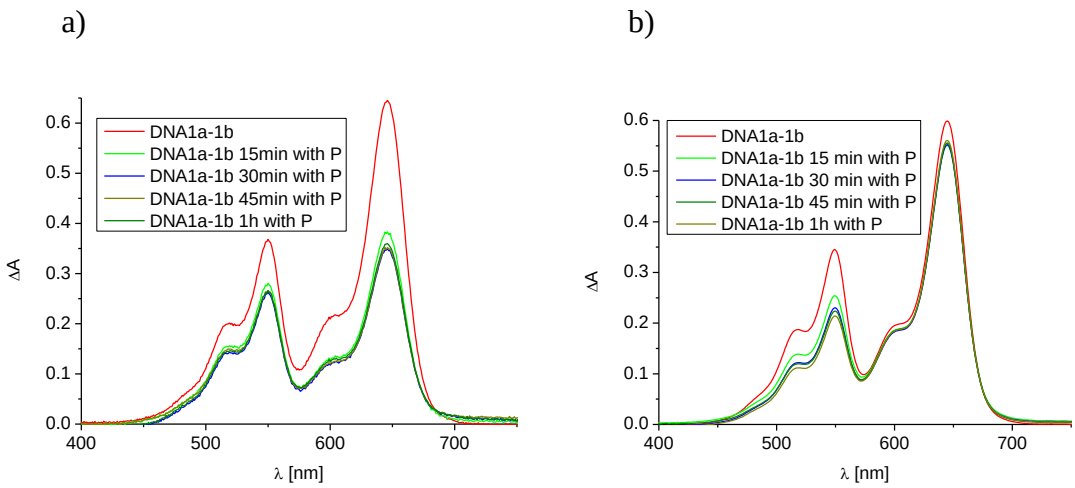


Figure S3: Measurements with a) **DNA0** and b) **PNA1** bearing particles.

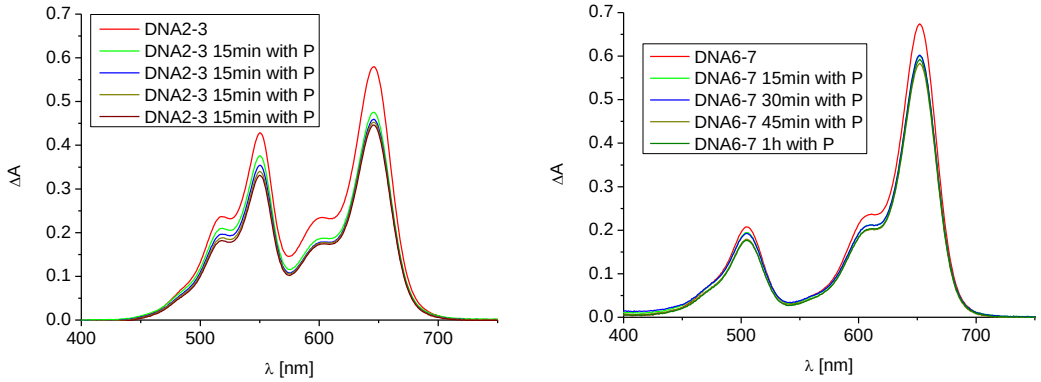


Figure S4: Measurements with **PNA2** bearing particles.

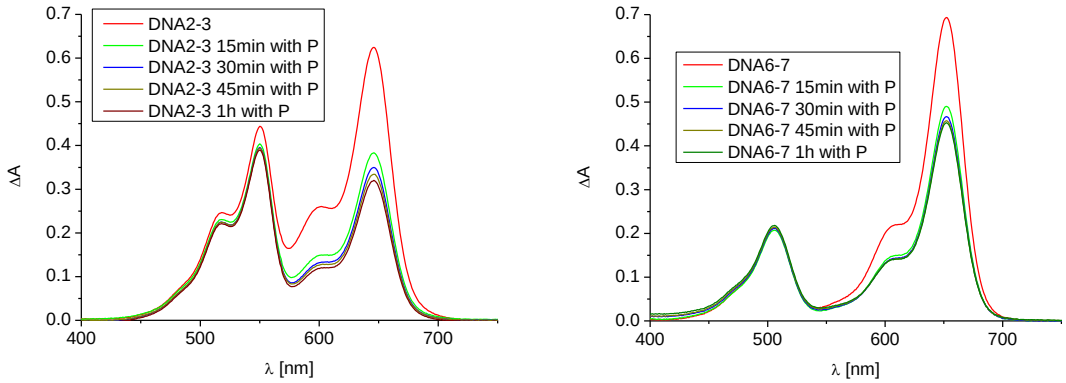


Figure S5: Measurements with **PNA3** bearing particles.

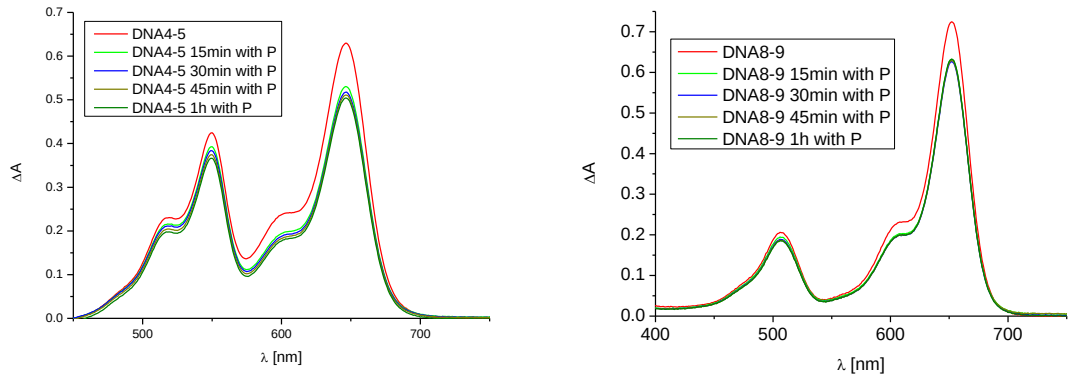


Figure S6: Measurements with **PNA4** bearing particles.

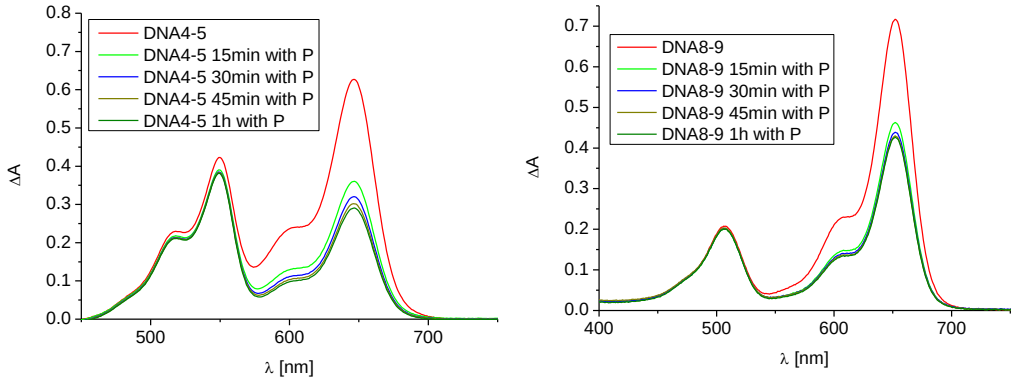


Figure S7: Measurements with **PNA5** bearing particles.

a) b)

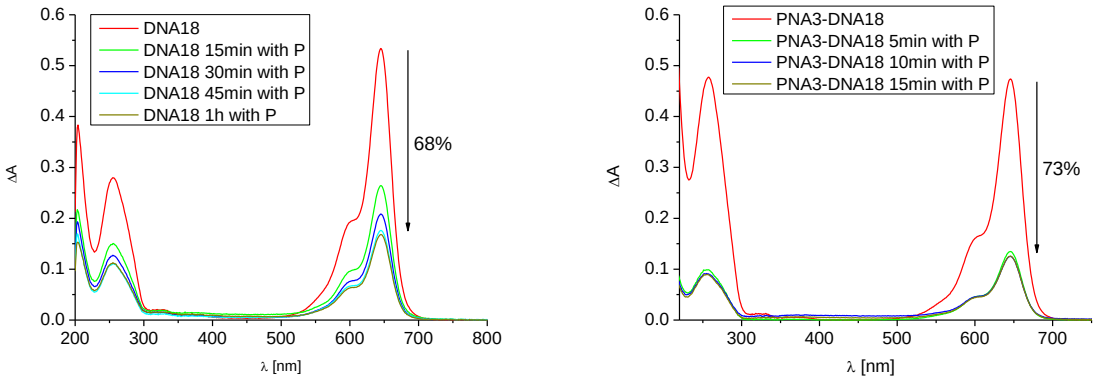


Figure S8: Hybridization control experiment of **DNA18** (5´-Cy5-GGGGCAGTGCCT-3´) and **PNA3**: (a) Equimolar hybridization of **DNA18** with **PNA3** bearing particles, (b) Hybrization of **DNA18** (1 equiv.) with excess of **PNA3** (1.2 equiv.) and subsequent immobilization on the streptavidin coated particles.

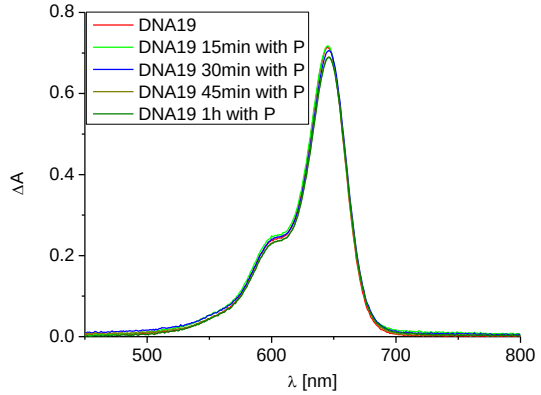


Figure S9: Control expertiment using „non-sense“ **DNA19** (5´-Cy5-GATAGTCTCATCGTC-3´) and magnetic paricles bearing non-complementary **DNA20** (5´-Biotin-GTACTGTGACTGATGCTGTGACGCA-3´).

4. References

[1] T. Vilaivan and C. Srisuwannaket, *Org. Lett.* **2006**, *8*, 1897.

[2] C. Vilaivan, C. Srisuwannaket, C. Ananthanawat, C. Suparpprom, J. Kawakami, Y. Yamaguchi, Y. Tanaka and T. Vilaivan, *Artificial DNA: PNA & XNA* **2011**, *2*, 50.