

Electronic Supplementary Information

Quenching of fluorescently labeled pyrrolidinyl peptide nucleic acid by oligodeoxyguanosine and its application in DNA sensing

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Entry	Caption	Page
Table S1	Sequence, isolated yield, and characterization data of PNA in this study	2
Table S2	Melting temperatures of PNA-DNA hybrids	2
Figure S1	Analytical HPLC chromatogram and MALDI-TOF mass spectrum of FluK1	3
Figure S2	Analytical HPLC chromatogram and MALDI-TOF mass spectrum of FluK5	4
Figure S3	Analytical HPLC chromatogram and MALDI-TOF mass spectrum of PNA1	5
Figure S4	Analytical HPLC chromatogram and MALDI-TOF mass spectrum of PNA2	6
Figure S5	Analytical HPLC chromatogram and MALDI-TOF mass spectrum of PNA3	7
Figure S6	Analytical HPLC chromatogram and MALDI-TOF mass spectrum of TMRK4	8
Figure S7	UV-visible spectra of FluK5 and its complexes with A) complementary DNA oligonucleotides bearing various 3' overhangs and B) homo-oligodeoxynucleotides	9
Figure S8	UV-visible spectra of FluK1 and FluK5 probes in comparison with free fluorescein and fluorescein-labeled DNA probe in the absence and presence of dG10	10
Figure S9	CD spectra of FluK5 and its complementary DNA oligonucleotides bearing various 3' overhangs A) before and B) after mixing	11
Figure S10	CD spectra of FluK5 and homo-oligodeoxynucleotides A) before and B) after mixing	12
Figure S11	CD spectra of various G-quadruplex-forming DNA sequences	13
Figure S12	Gel electrophoresis experiments A) visualized under transilluminator (365 nm) B) visualized by UV shadowing (256 nm) over a silica gel GF254-coated TLC plate	13
Figure S13	Brightness percentage of FluK5 PNA probe-dG10 complex at 525 nm in the presence of various quantities of complementary (dCs) and non-complementary DNA targets	14
Figure S14	Brightness percentage of PNA-DNA hybrids deriving from fluorescein- and TMR-labeled PNA probes	14
Figure S15	Raw fluorescence spectra of fluorescein- and TMR-labeled PNA probes and their DNA hybrids	15

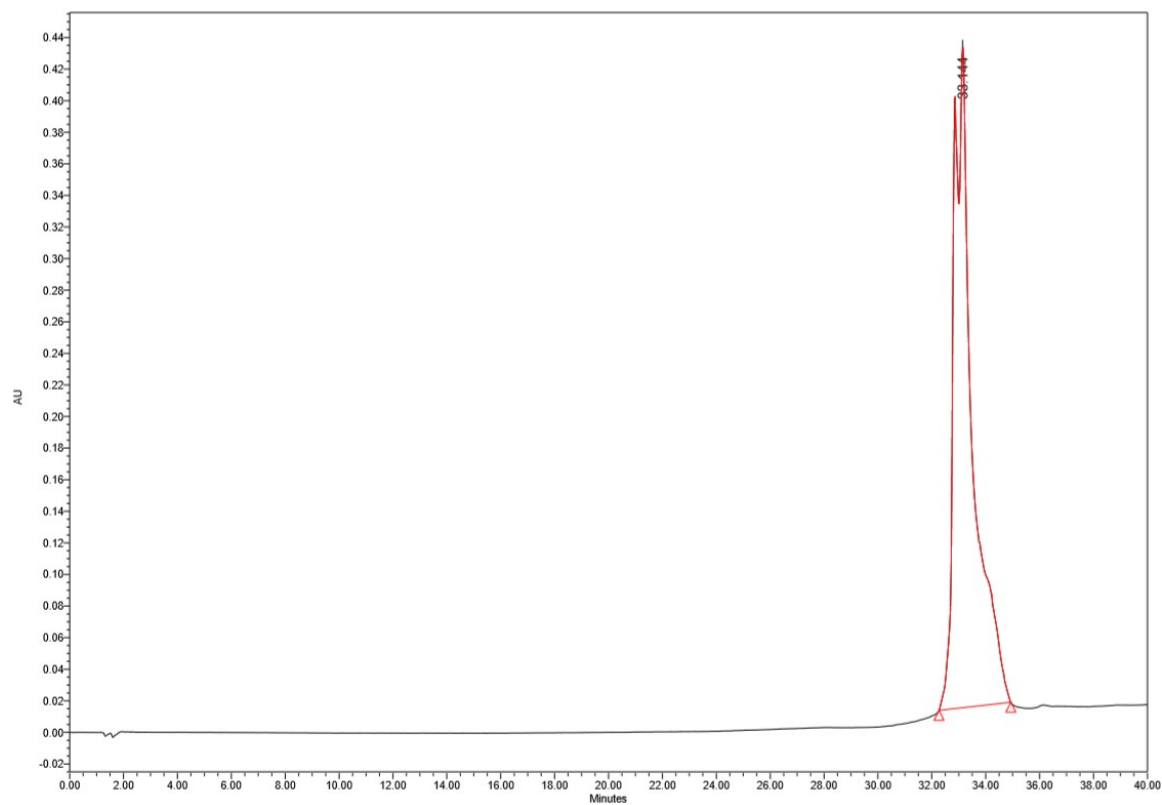
Table S1. Sequence, isolated yield and characterization data of PNA in this study

PNA	Sequence (N→C)	Isolated yield (%) ^a	<i>m/z</i> calcd	<i>m/z</i> found
FluK1	Flu-GTAGATCACT-K	25	3875.10	3879.48
FluK5	Flu-GTAGATCACT-KKKKK	30	4387.79	4385.69
PNA1	Flu-GCTTTTTTACA-KKKKK	21	4686.12	4685.36
PNA2	Flu-AGTCTGATAAGC-KKKKK	17	5086.53	5085.49
PNA3	Flu-TTAATACCTTTGCTC-KKK	23	5738.22	5737.36
TMRK4	TMR-CTAAATTCAGA-KKKK	18	4640.13	4637.77

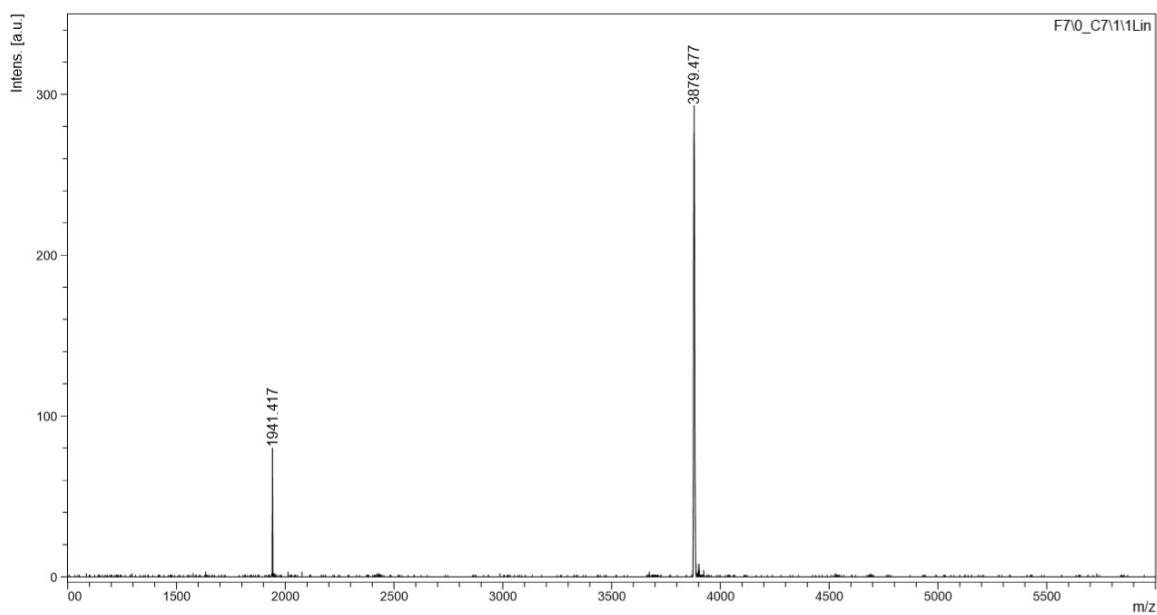
^a Isolated yield after HPLC purification, spectrophotometrically determined

Table S2. Melting temperatures of PNA-DNA hybrids. Conditions: [PNA] = 1 μM, [DNA] = 1.2 μM in 10 mM phosphate buffer pH 7.0. The probe binding region in the DNA targets is underlined.

PNA	DNA	Sequence (N→C)	<i>T_m</i> (°C)
FluK1		Flu-GTAGATCACT-K	
	dCs/dFK5	3'- <u>CATCTAGTGA</u> -5'	47.6
FluK5		Flu-GTAGATCACT-KKKKK	
	dCs/dFK5	3'- <u>CATCTAGTGA</u> -5'	71.3
	dMs	3'- <u>CATCTCGTGA</u> -5'	39.7
	dCL	3'-GGTCCCGTACC <u>ATCTAGTGACATGCGGCCGC</u> -5'	82.5
	dML	3'-GGTCCCGTACC <u>ATCTCGTGACATGCGGCCGC</u> -5'	59.2
PNA1		Flu-GCTTTTTTACA-KKKKK	
	DNA1	3'- <u>CGAAAAAATGT</u> -5'	74.1
PNA2		Flu-AGTCTGATAAGC-KKKKK	
	DNA2	3'-AGTTGTAGT <u>CAGACTATTCGAT</u> -5'	76.7
PNA3		Flu-TTAATACCTTTGCTC-KKK	
	DNA3	3'- <u>AATTATGGAAACGAG</u> -5'	>95
TMRK4		TMR-CTAAATTCAGA-KKKK	
	dTK4	3'- <u>AATTTAAGTCT</u> -5'	84.5

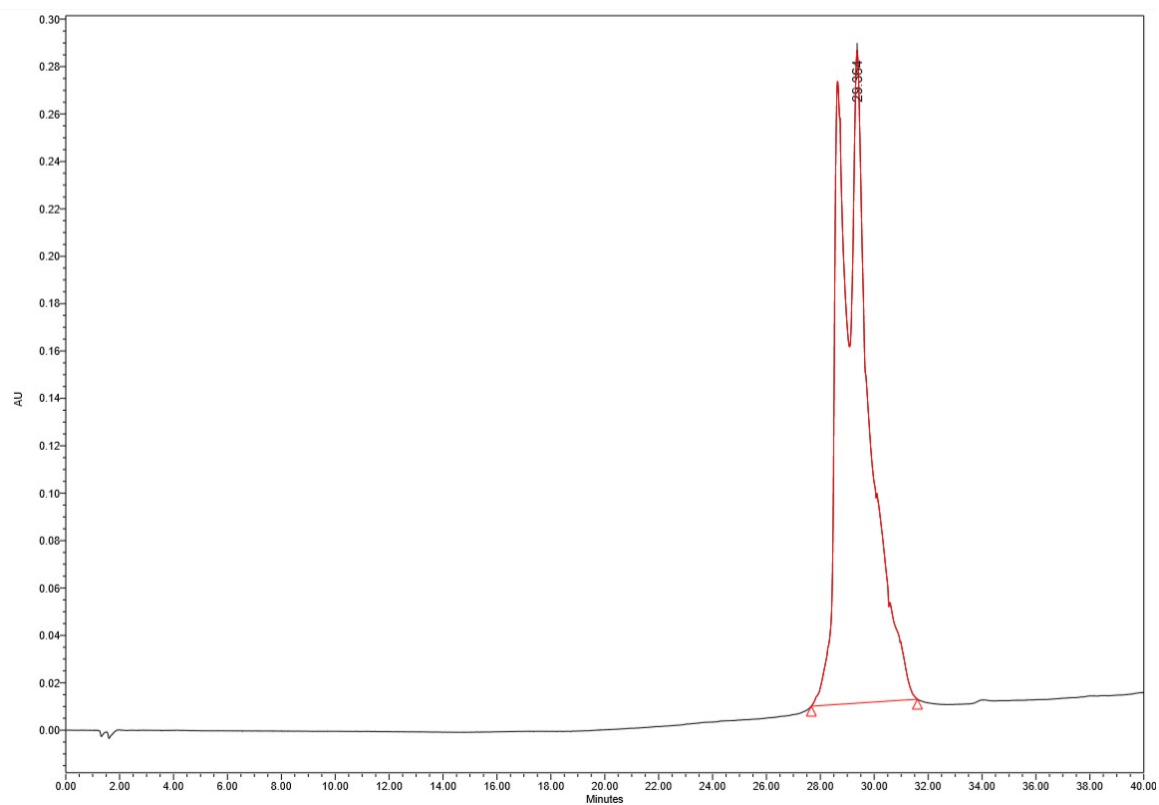


A

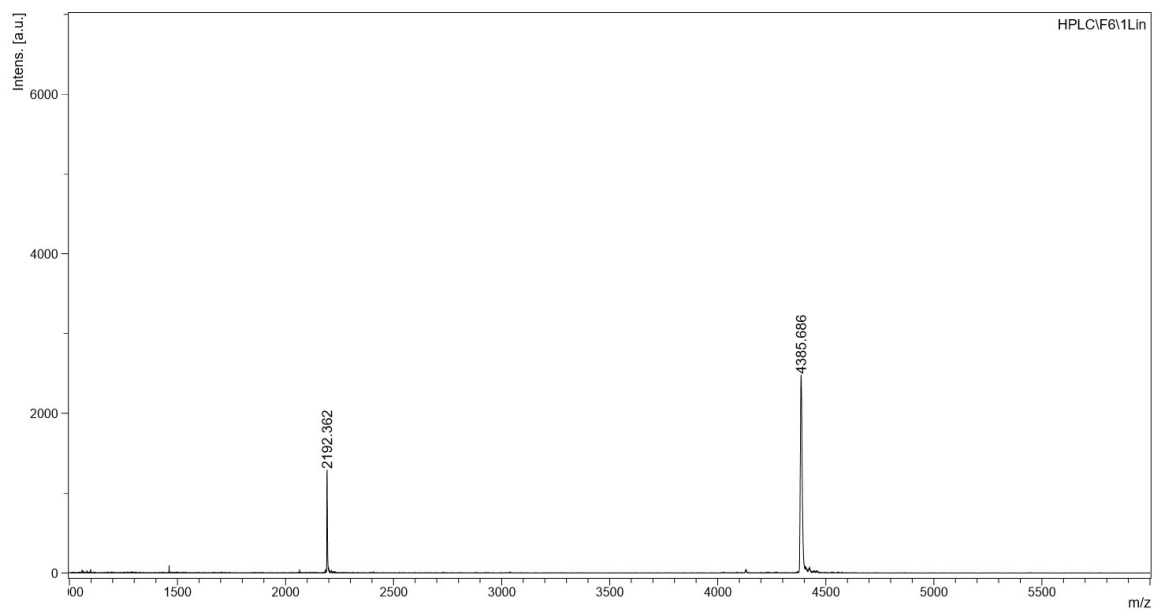


B

Figure S1. A) Analytical HPLC chromatogram and B) MALDI-TOF mass spectrum of FluK1 (calcd for $[M \cdot H]^+ = 3875.10$). The two peaks in the HPLC chromatogram are attributed to the presence of two isomers (5- and 6-) of the carboxyfluorescein label.

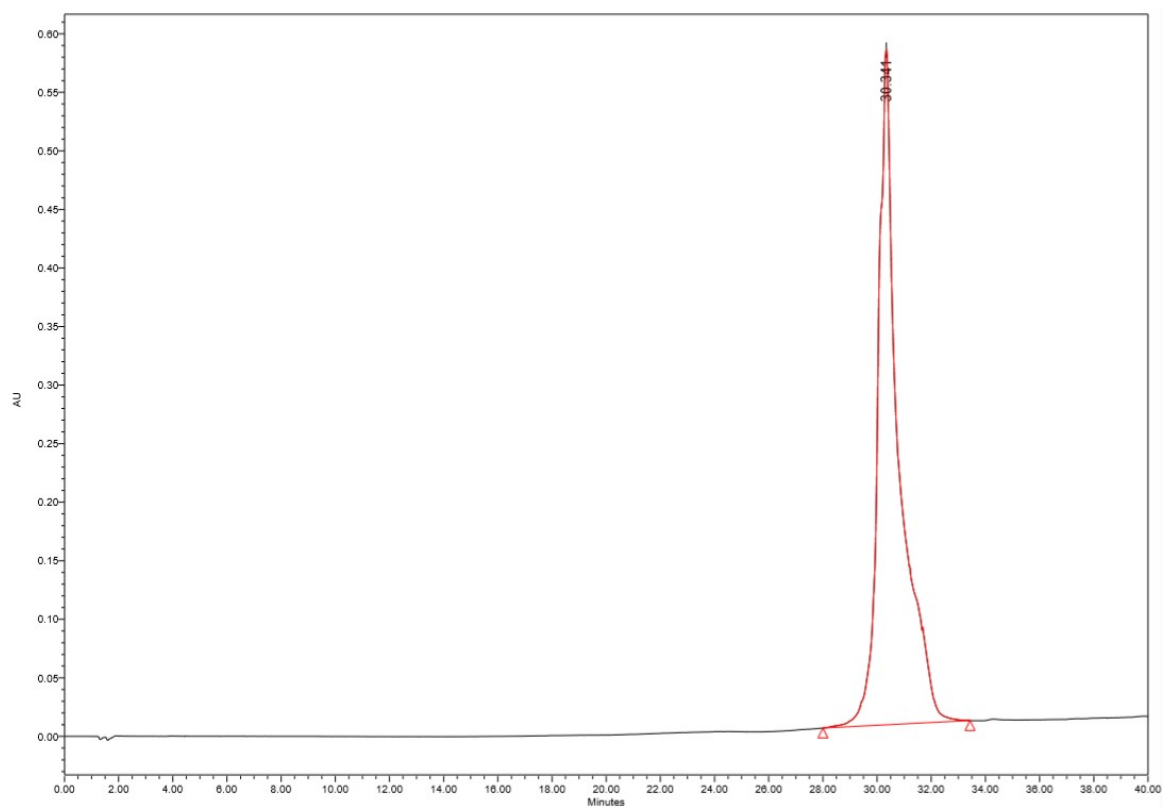


A

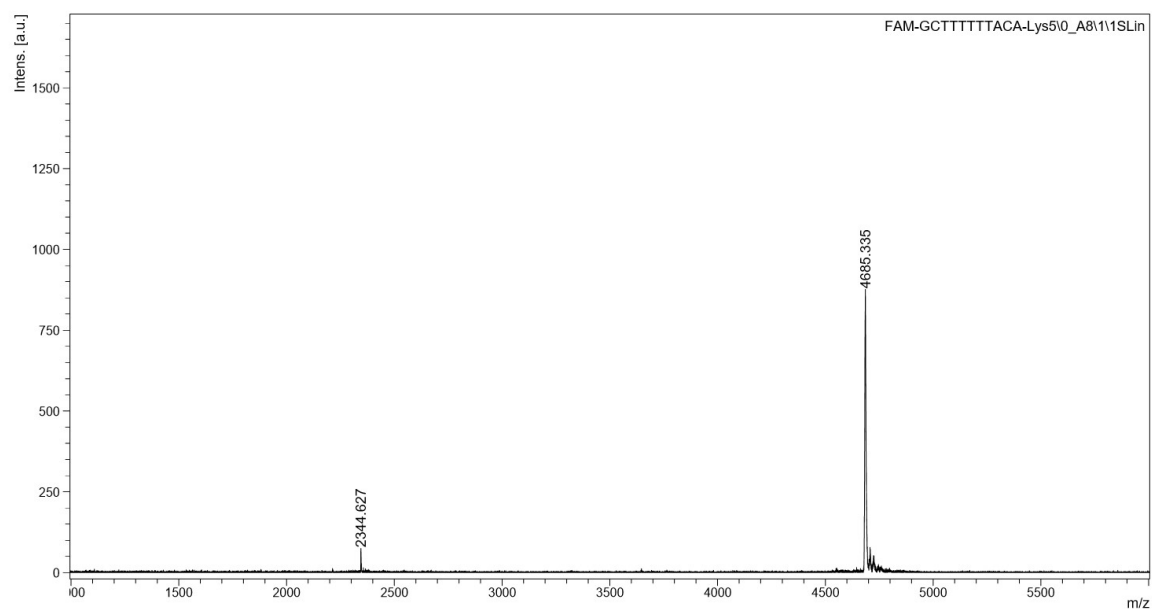


B

Figure S2. A) Analytical HPLC chromatogram and B) MALDI-TOF mass spectrum of FluK5 (calcd for $[M \cdot H]^+ = 4387.79$). The two peaks in the HPLC chromatogram are attributed to the presence of two isomers (5- and 6-) of the carboxyfluorescein label.

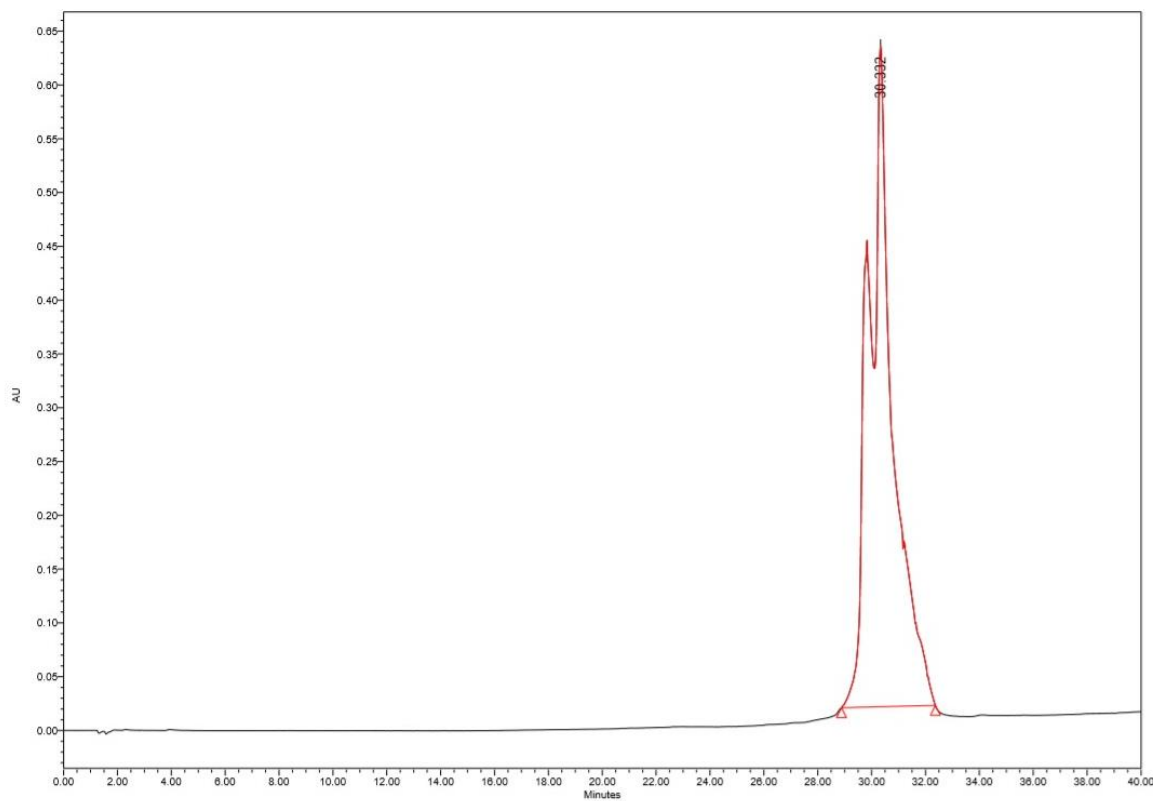


A

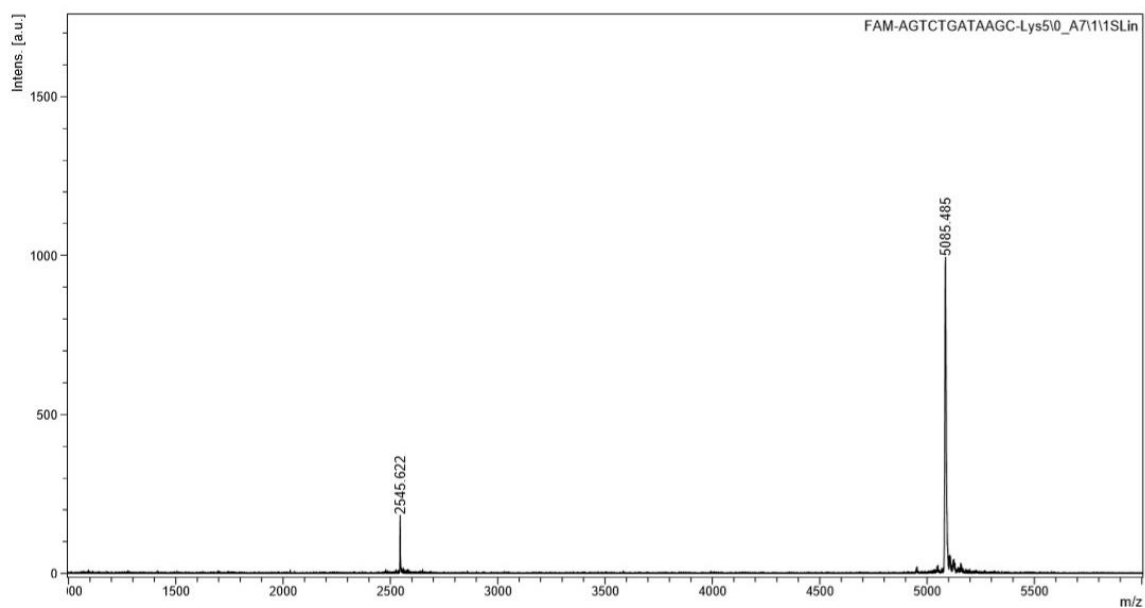


B

Figure S3. A) Analytical HPLC chromatogram and B) MALDI-TOF mass spectrum of PNA1 (calcd for $[M \cdot H]^+ = 4686.12$)

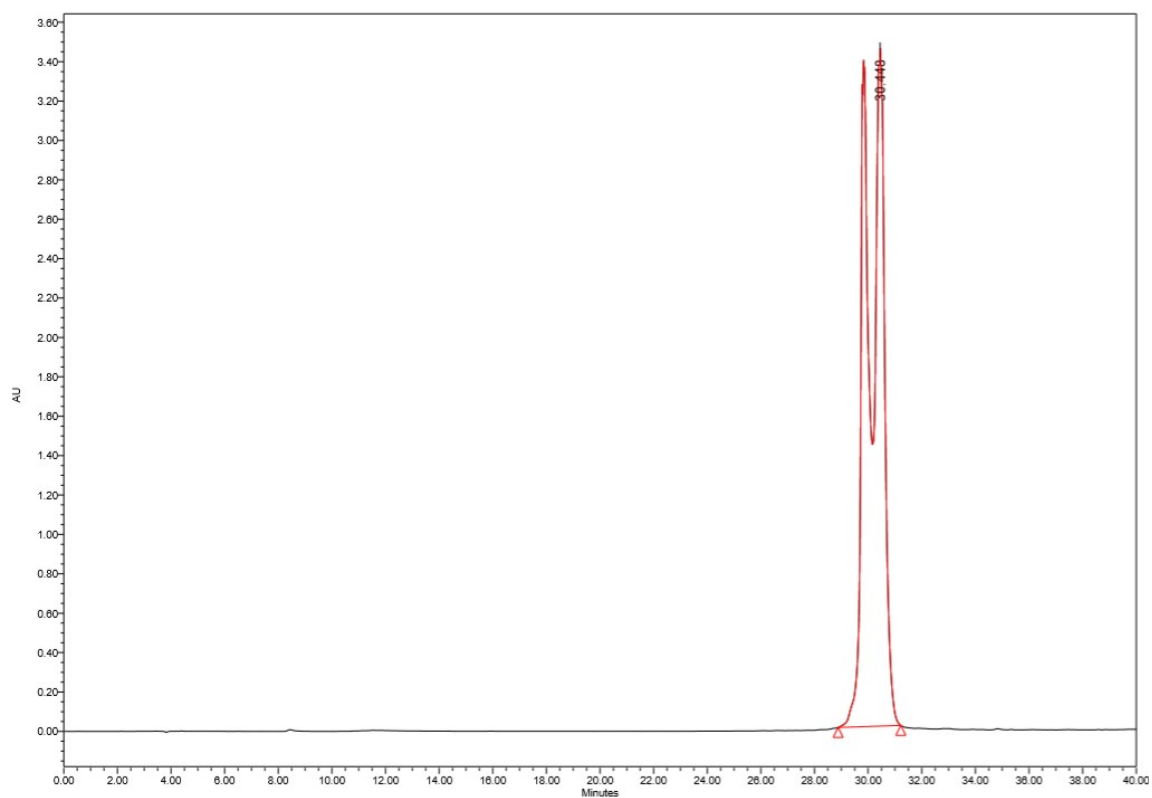


A

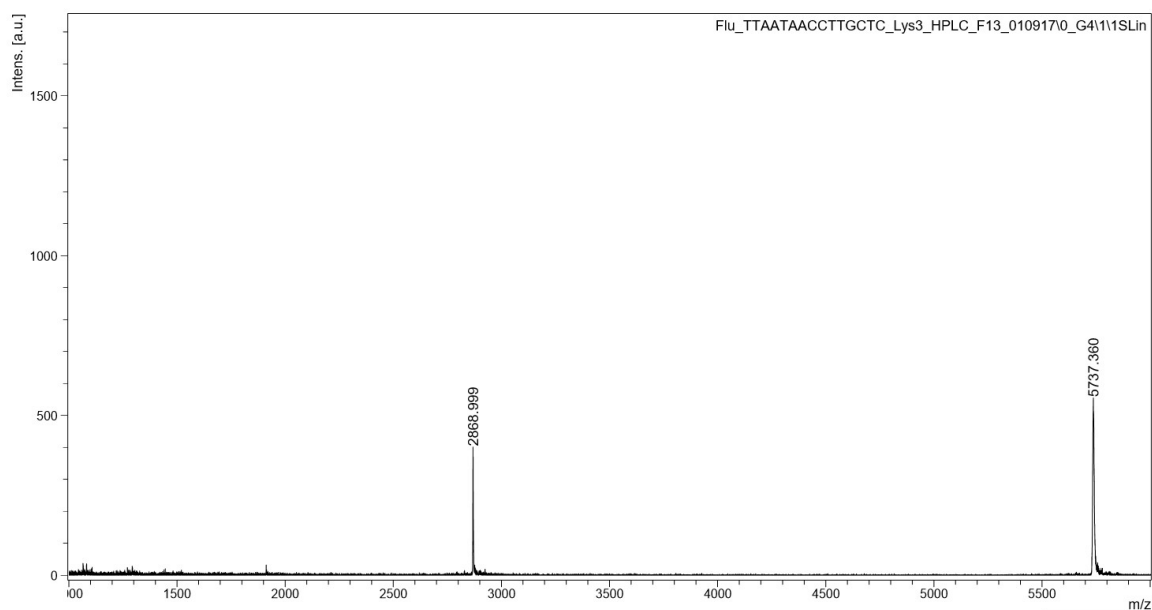


B

Figure S4. A) Analytical HPLC chromatogram and B) MALDI-TOF mass spectrum of PNA2 (calcd for $[M \cdot H]^+ = 5086.53$). The two peaks in the HPLC chromatogram are attributed to the presence of two isomers (5- and 6-) of the carboxyfluorescein label.

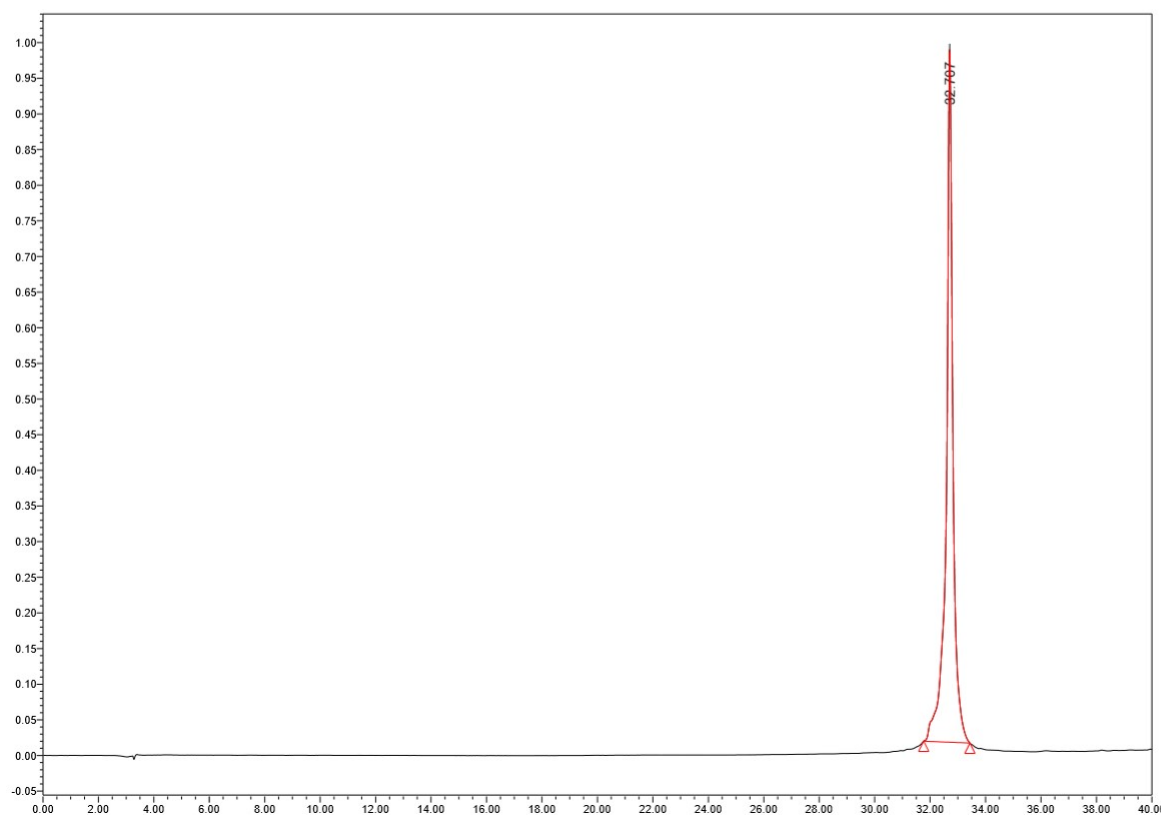


A

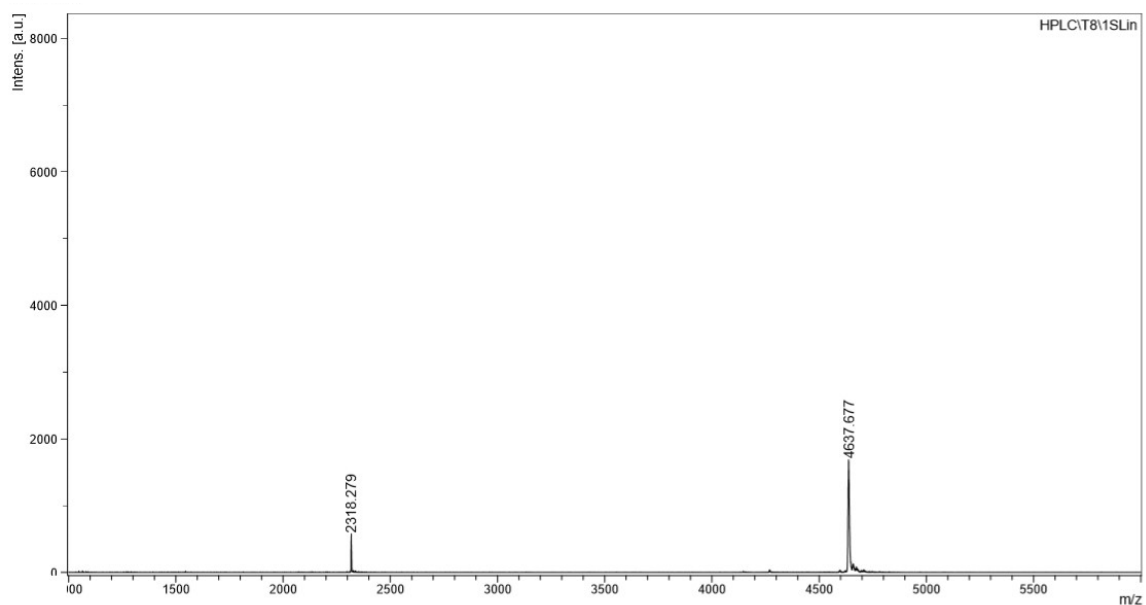


B

Figure S5. A) Analytical HPLC chromatogram and B) MALDI-TOF mass spectrum of PNA3 (calcd for $[M \cdot H]^+ = 5738.18$). The two peaks in the HPLC chromatogram are attributed to the presence of two isomers (5- and 6-) of the carboxyfluorescein label.

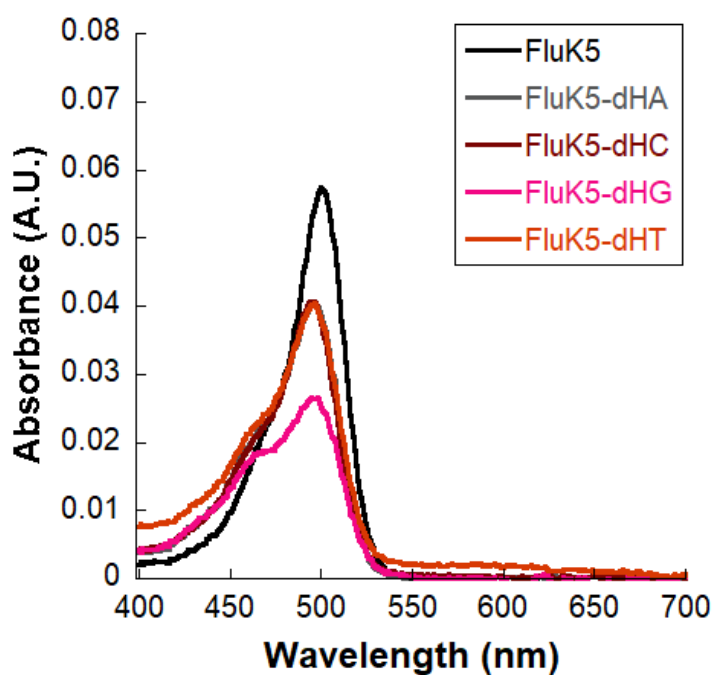


A

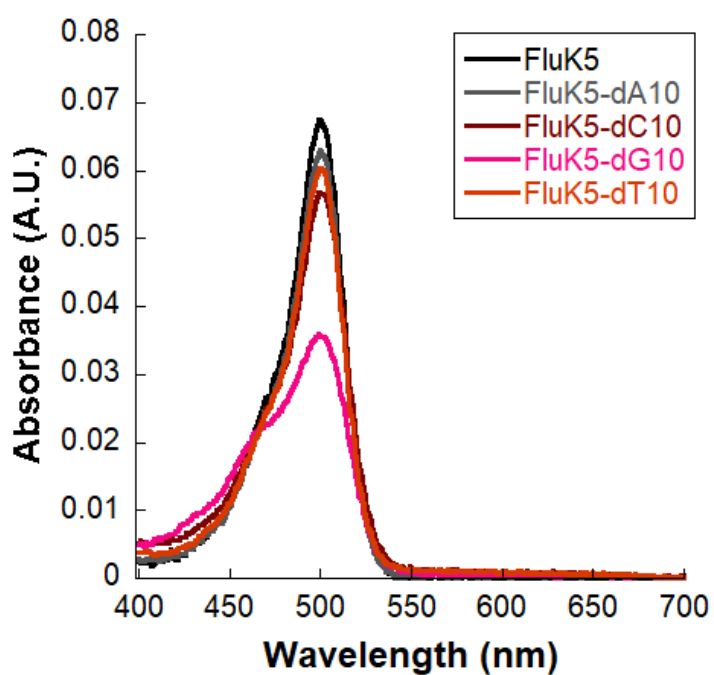


B

Figure S6. A) Analytical HPLC chromatogram and B) MALDI-TOF mass spectrum of TMRK4 (calcd for $[M \cdot H]^+ = 4640.13$)



A



B

Figure S7. UV-visible spectra of FluK5 and its complexes with A) complementary DNA oligonucleotides bearing various 3' overhangs and B) homo-oligodeoxynucleotides. Conditions: [FluK5] = 1.0 μ M, [DNA] = 1.2 μ M in 10 mM sodium phosphate buffer pH 7.0.

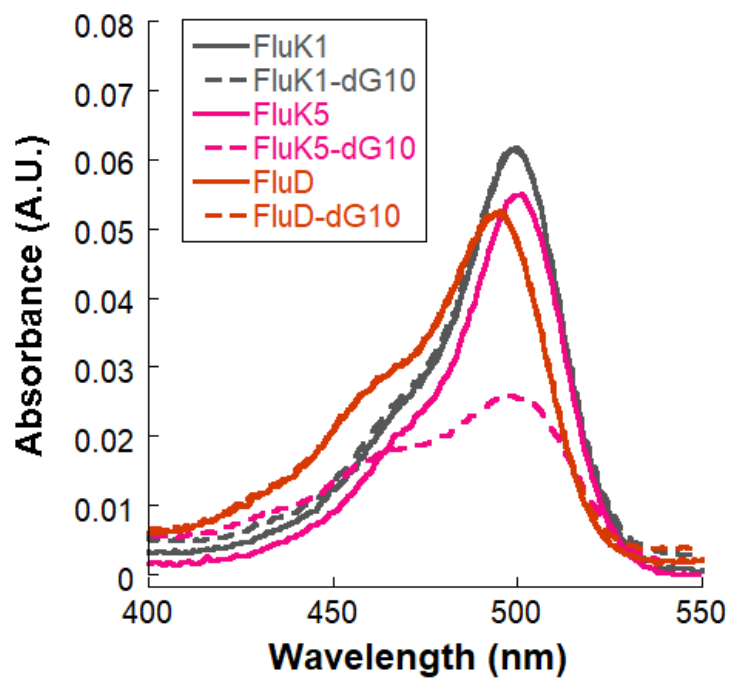
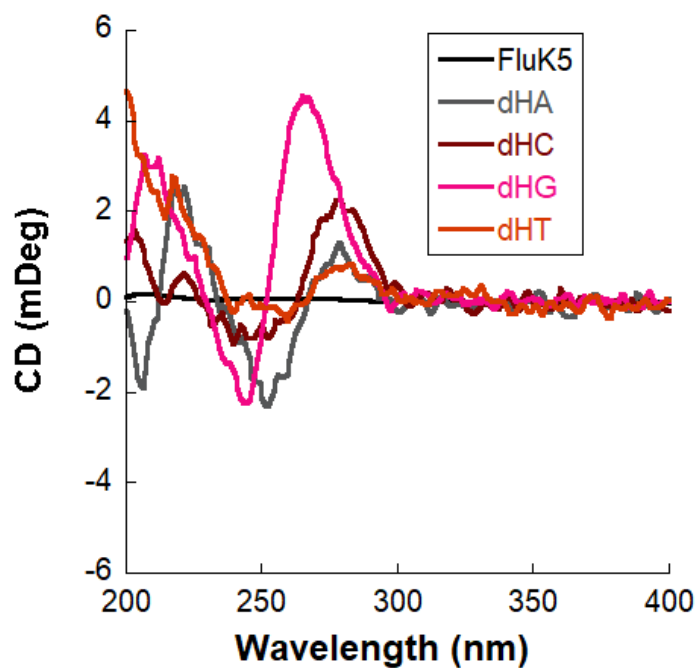
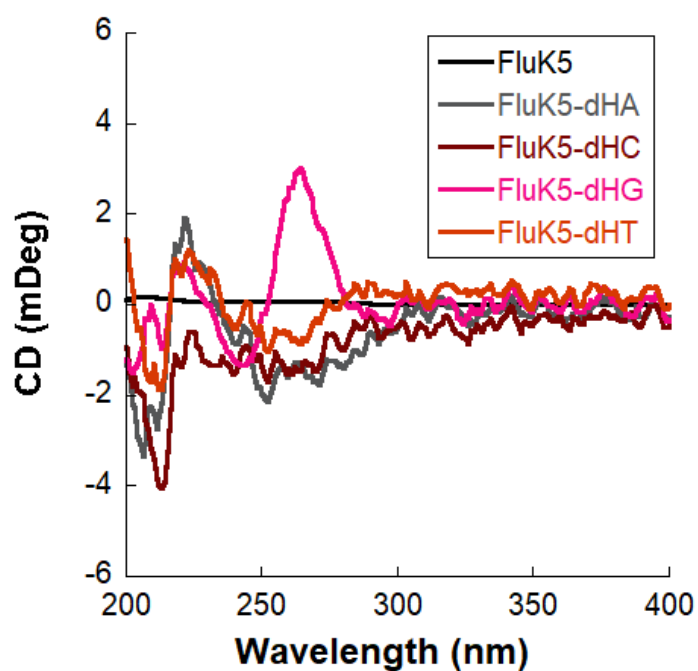


Figure S8. UV-visible spectra of FluK1 and FluK5 probes in comparison with free fluorescein and fluorescein-labeled DNA probe in the absence and presence of dG10. Conditions: [Probe] = 1.0 μM , [DNA] = 1.2 μM in 10 mM sodium phosphate buffer pH 7.0.

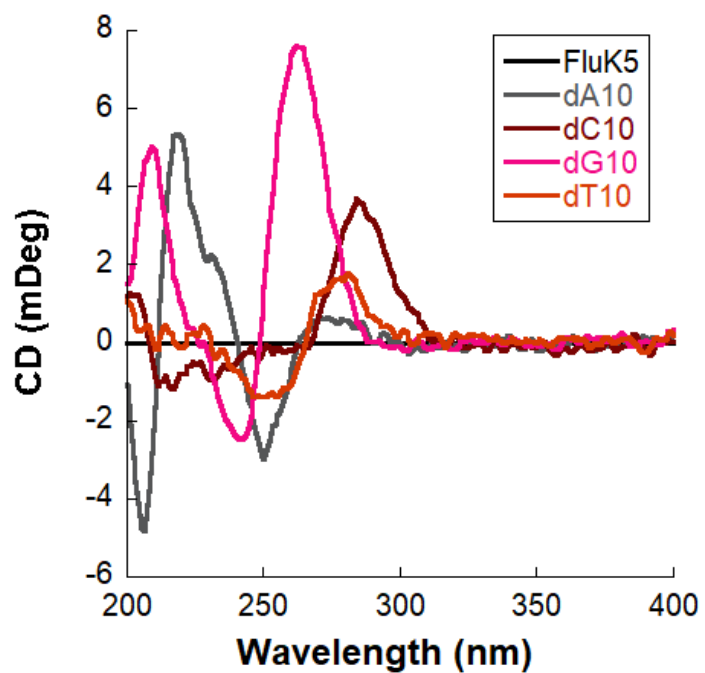


A

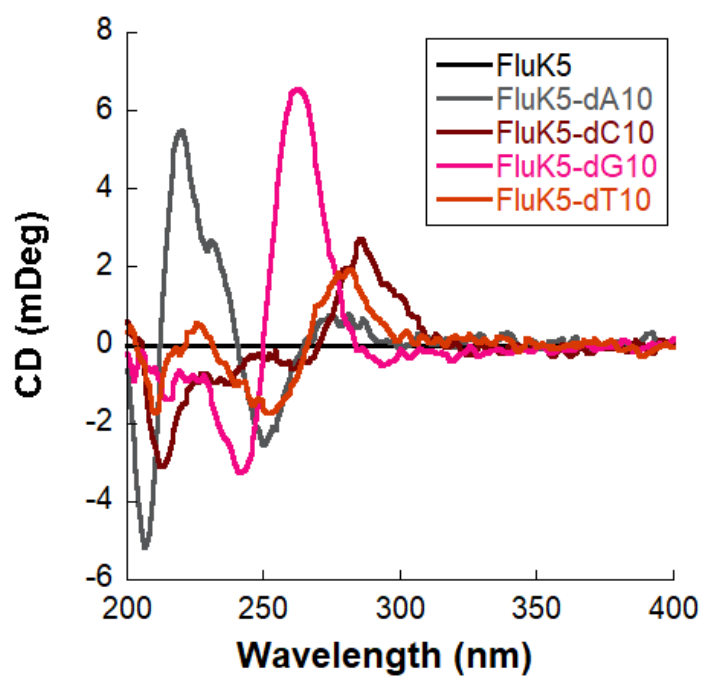


B

Figure S9. CD spectra of FluK5 and its complementary DNA oligonucleotides bearing various 3' overhangs A) before and B) after mixing. Conditions: [FluK5] = 1.0 μ M, [DNA] = 1.2 μ M in 10 mM sodium phosphate buffer pH 7.0.



A



B

Figure S10. CD spectra of FluK5 and homo-oligodeoxynucleotides A) before and B) after mixing. Conditions: [FluK5] = 1.0 μ M, [DNA] = 1.2 μ M in 10 mM sodium phosphate buffer pH 7.0.

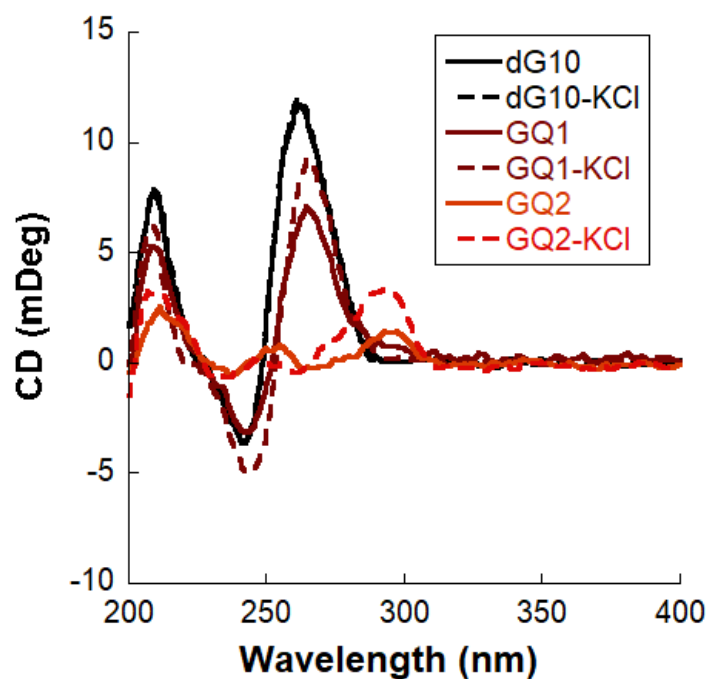


Figure S11. CD spectra of various G-quadruplex-forming DNA sequences. Conditions: [DNA] = 1.2 μ M, [KCl] = 0 or 10 mM in 10 mM sodium phosphate buffer pH 7.0.

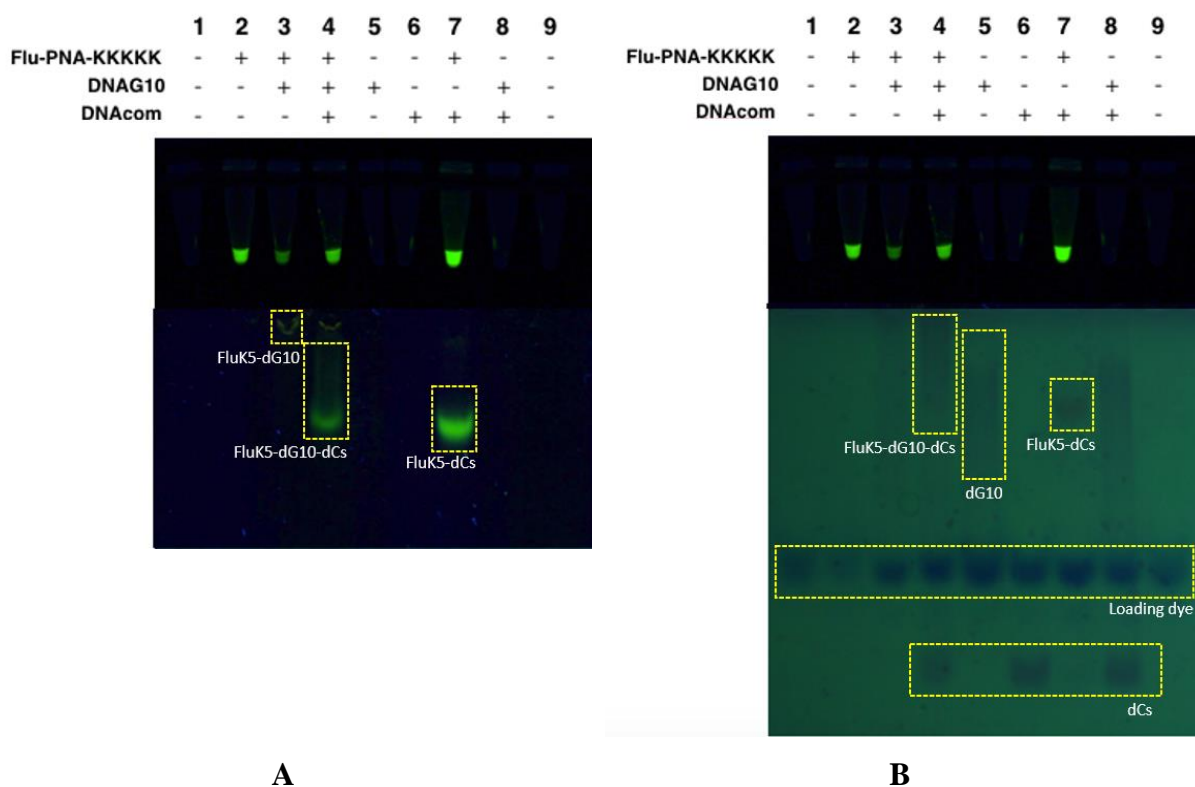


Figure S12. Gel electrophoresis experiments A) visualized under transilluminator (365 nm) B) visualized by UV shadowing (256 nm) over a silica gel GF254-coated TLC plate. The amounts of PNA and DNA (dCs/dG10) in each lane are 0.5 and 0.6 nmol, respectively.

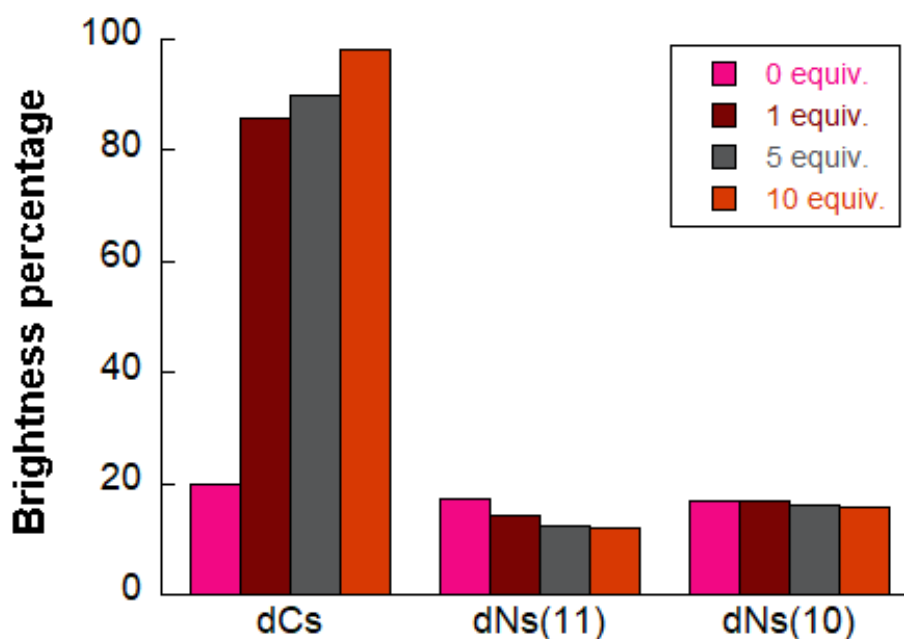


Figure S13. Brightness percentage of FluK5-dG10 complex at 525 nm in the presence of various quantities of complementary (dCs) and non-complementary DNA targets dNs(11): 5'-TCTGAATTTAA-3' and dNs(10): 5'-GTAGATCACT-3'. Conditions: [FluK5] = [dG10] = 0.1 μ M, [DNA] = 0.1 – 1.0 μ M in 10 mM sodium phosphate buffer pH 7.0.

*Brightness percentage = $(F_{\text{probe+dG10+DNA}})/F_{\text{probe}} * 100$

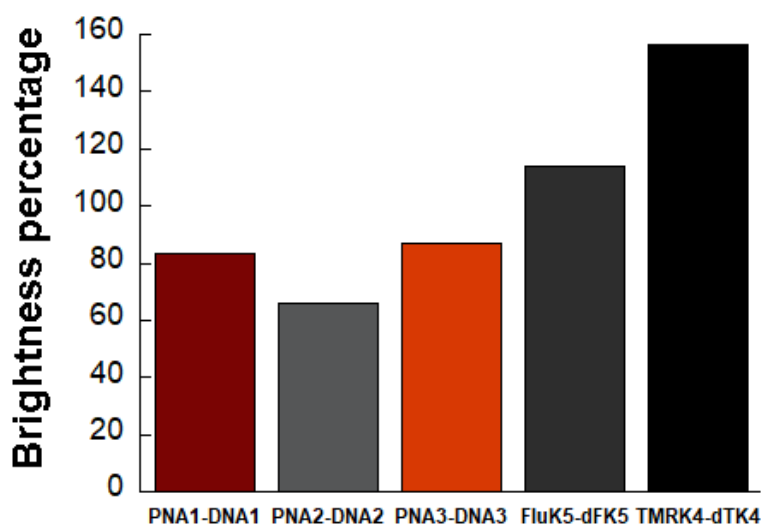


Figure S14. Brightness percentage of PNA-DNA duplexes of Flu-labeled PNA probes (PNA1, PNA2, PNA3, and FluK5) (λ_{ex} 490 nm, λ_{em} 525 nm) and a TMR-labeled PNA probe (TMRK4) (λ_{ex} 520 nm, λ_{em} 555 nm). Conditions: [PNA] = 0.1 μ M, [DNA] = 0.12 μ M in 10 mM sodium phosphate buffer pH 7.0.

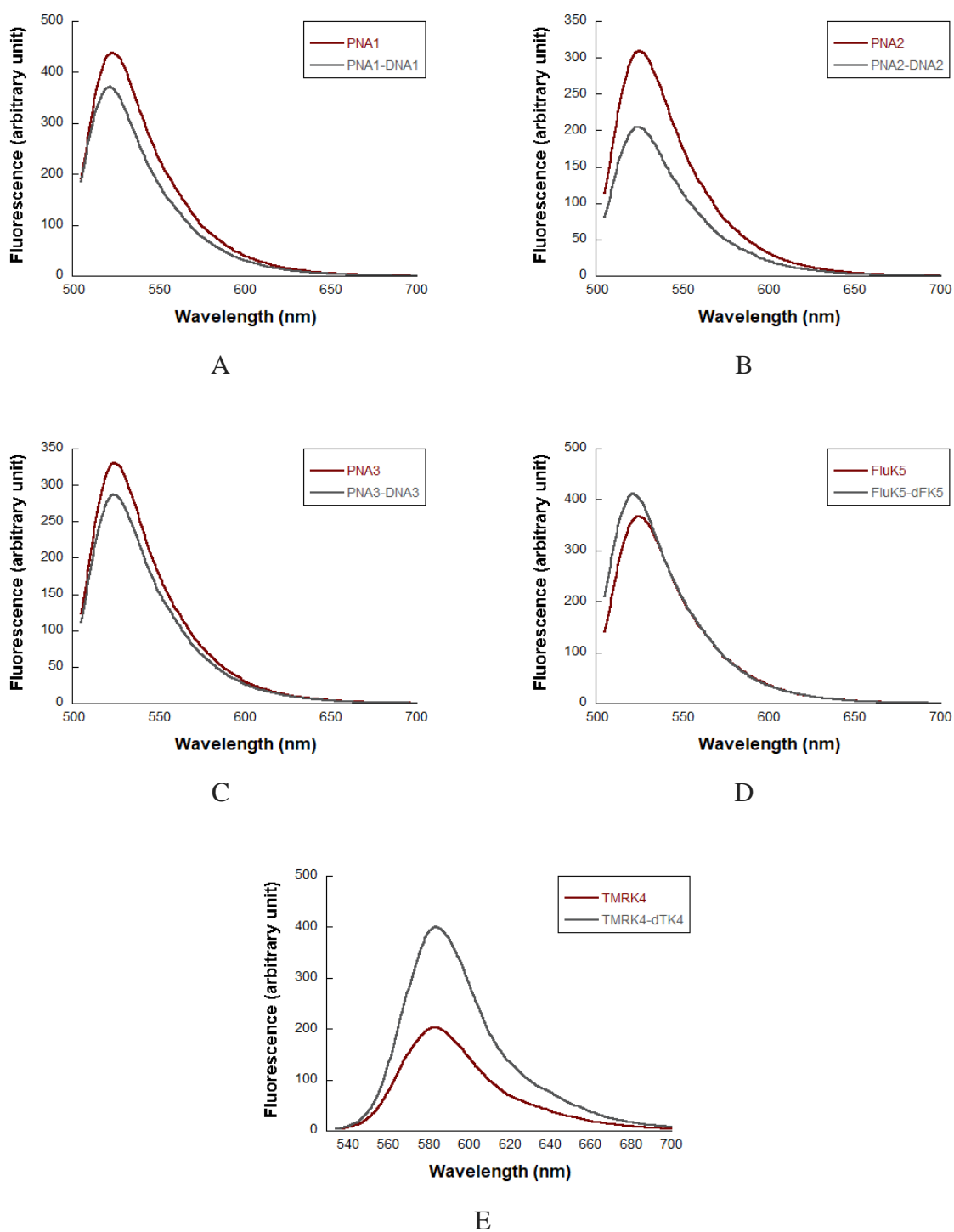


Figure S15. Raw fluorescence spectra of fluorescein-labeled PNA probes (A: PNA1, B: PNA2, C: PNA3, and D: FluK5) (λ_{ex} 490 nm, λ_{em} 525 nm) and a TMR-labeled PNA probe (E: TMRK4) (λ_{ex} 520 nm, λ_{em} 555 nm) and their DNA hybrids. Conditions: [PNA] = 0.1 μM , [DNA] = 0.12 μM in 10 mM sodium phosphate buffer pH 7.0.