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Cyclopentane FIT-PNAs: Bright RNA sensors

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General procedures and materials

Manual solid-phase synthesis was performed by using 5 mL polyethylene syringe reactors (Phenomenex) that are equipped with a fritted disk. HPLC purifications and analysis were performed on a Shimadzu LC-1090 system using a semi-preparative C18 reversed-phase column (Jupiter C18, 5u, 300Å, 250x10mm, Phenomenex) at 50ºC. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient with a flow rate of 4mL/min. Mass analysis of FIT-PNAs was acquired on a TSQ Quantum Access Max (Thermo Fisher Scientifc, Basel, Switzerland) mass spectrometer. The analysis was performed by direct injection into the mass spectrometer using electrospray ionization (ESI) in positive mode and full scan analysis (range of 200–1500 m/z).

RNA oligos were purchased from IDT, USA. Fmoc/Bhoc protected PNA monomers from PolyOrg Inc. (USA). Fmoc-D-Lysine and reagents for solid phase synthesis were purchased from Merck (Germany) and Biolab (Israel). Fmoc-protected cyclopentane PNA monomers (C and T) and BisQ were synthesized as previously reported.[1,2]

Solid phase synthesis of cpFIT-PNA and FIT-PNA

Coupling of D-Lysine onto Novasyn TGA Resin. The resin (250 mg, 0.2 mmol/g) was allowed to swell in 10 ml DMF for 30 min. For pre-activation, DIC (5 eq.) and DIMAP (0.1eq.) were added to a solution of Fmoc-protected D-Lysine (10eq.) in DCM (15 ml) in an ice bath. After 15 min, the mixture was evaporated, re-dissolved in dry DMF and added to the resin. After 2.5 h, the resin was washed with DMF (5x2 mL), CH_2Cl_2 (5x2 mL) and the procedure was repeated.

Fmoc Cleavage. A solution of DMF/piperidine (4:1, 1 ml) was added to the resin. After 2 min the procedure was repeated. Finally, the resin was washed with DMF (3x1 ml), DCM (3x1 ml).

Coupling of Fmoc-Bhoc-PNA-Monomers. 4eq. of PNA monomer, 4eq. HATU, 4eq. HOBt and 8eq. of dry DIPEA in DMF (to 0.1 M PNA) were mixed in a glass vial equipped with a screw cap. After 3 min of pre-activation, the solution was transferred to the resin. After 60 min, the reaction mixture was discarded and the resin was washed with DMF (2x1 ml) and DCM (2x1 ml).

Coupling of BisQ. 4eq. of BisQ monomer, 4eq. HATU, 4eq. HOBt and 8eq. of dry DIPEA in DMF (to 0.1 M BisQ monomer) were mixed in a glass vial equipped with screw cap. Following 3 min of pre-activation, the solution was transferred to the resin.

After 60 min, the procedure was repeated and finally the resin was washed with DMF (2x1 ml) and DCM (2x1 ml).

Coupling of cyclopentane modified PNAs. 4eq. of cpPNA monomer, 4eq. HATU, 4eq. HOBt and 8eq. of dry DIPEA in DMF (to 0.1 M cpPNA) were mixed in a glass vial equipped with screw cap. Following 3 min of pre-activation, the solution was transferred to the resin and shaked for 135 min. Finally, the resin was washed with DMF (2x1 ml) and DCM (2x1 ml).

Cleavage of PNA from resin. 1ml TFA was added to the dry resin. After 2h another portion of TFA was added. The combined TFA solutions were concentrated in vacuo.

PNA Purification. PNAs were precipitated from the concentrated TFA solution by addition of cold diethyl ether (10 ml). The precipitate was collected by centrifugation and decantation of the supernatant. The residue was dissolved in water and purified by semi preparative HPLC. The purified PNAs were analysed by ESI-MS.

Fluorescence spectrometry

Fluorescence spectra were recorded by using a Jasco FT-6500 spectrometer. Measurements were carried out in fluorescence quartz cuvettes (10 mm) at a 3 μM concentration of FIT-PNA in a PBS buffered solution (100 mM NaCl, 10 mM NaH_2PO_4, pH 7). Quantum yields were determined relative to Cresyl Violet in PBS.[3] PNAs were hybridized to complementary RNA using a 1:1.5 mixture of PNA:RNA at 37 ºC for 1-2 hr. Samples were excited at 575 nm and emission spectra were recorded at 585-800 nm.

Fluorescence end point were recorded by using a Cytation 3 plate reader. Measurements were carried out in Greiner 96 well black plates with flat bottom, at 0.5 μM concentration in a PBS buffered solution (100 mM NaCl, 10 mM NaH_2PO_4, pH 7). FIT-PNAs were hybridized to complementary RNA using a 1:1.5 mixture of PNA:RNA at 37 ºC for 1-2 hr or by allowing overnight incubation at RT (for limit of detection (LOD) measurements). Samples were excited at 575 nm and measured at 615 nm.

HPLC and MS of FIT-PNAs 1-7:

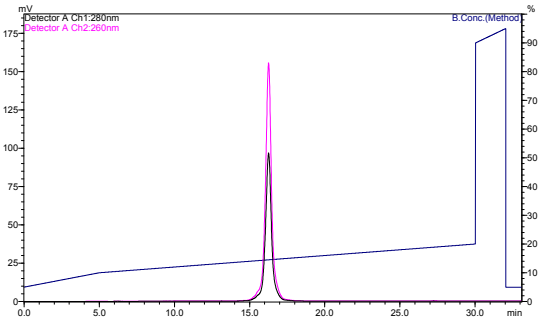

Fig. S1: HPLC chromatogram of FIT-PNA **1**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

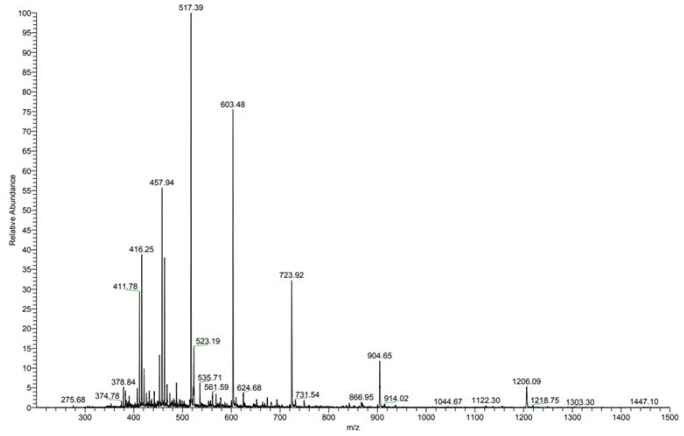

Fig. S2: ESI-MS of FIT-PNA **1**. M_{calc} = 3618.77, M_{obs} =3614.6.

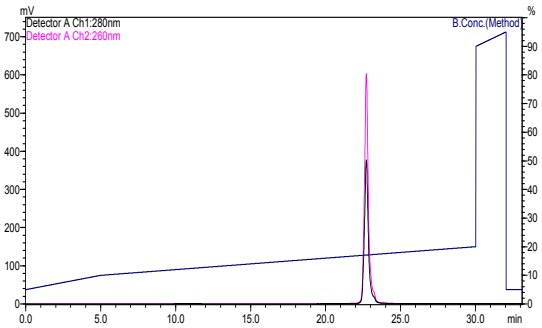

Fig. S3: HPLC chromatogram of FIT-PNA **2**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

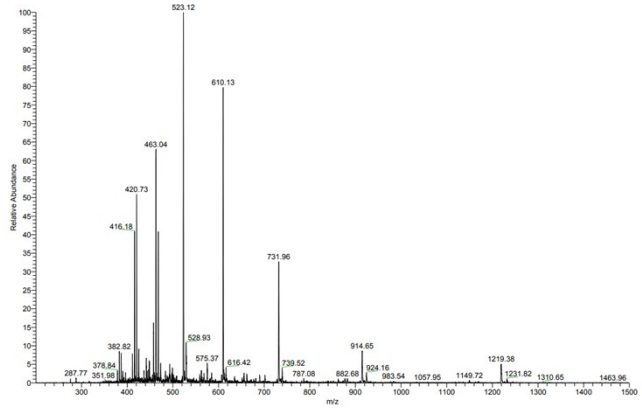

Fig. S4: ESI-MS of FIT-PNA **2**. M_{calc} = 3658.84, M_{obs} =3654.8.

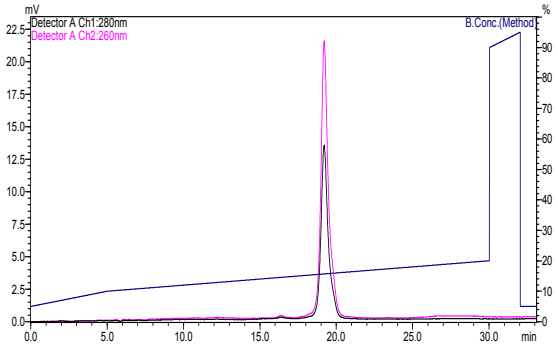

Fig. S5: HPLC chromatogram of FIT-PNA **3**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

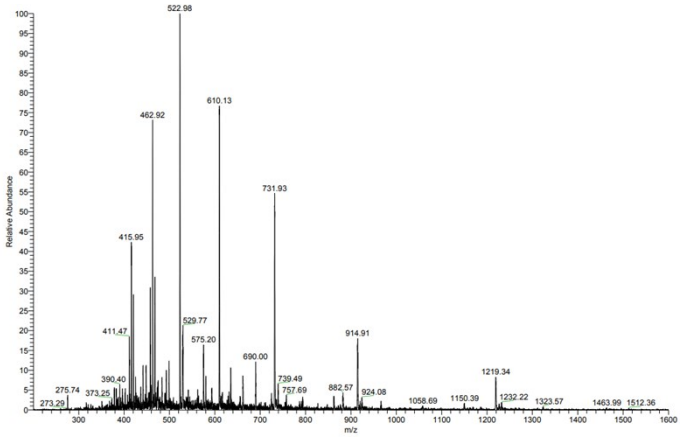

Fig. S6: ESI-MS of FIT-PNA **3**. M_{calc} = 3658.84, M_{obs} =3654.8.

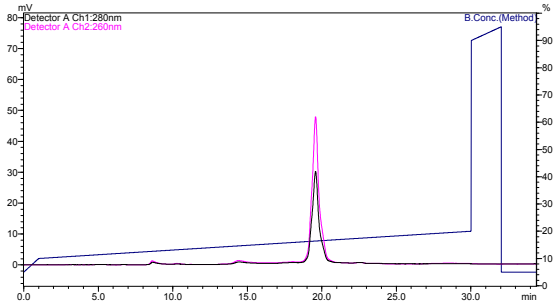

Fig. S7: HPLC chromatogram of FIT-PNA **4**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

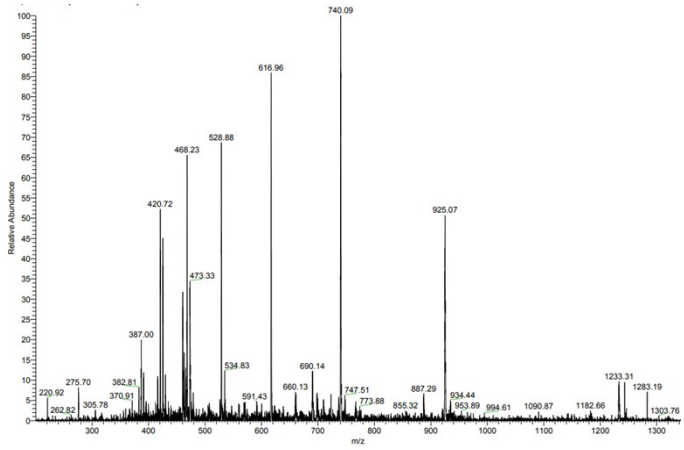

Fig. S8: ESI-MS of FIT-PNA **4**. M_{calc} = 3698.9, M_{obs} =3695.9.

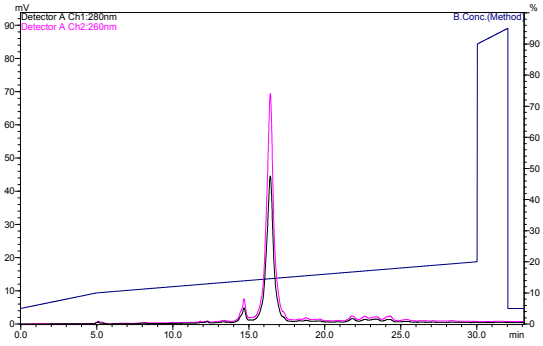

Fig. S9: HPLC chromatogram of FIT-PNA **5**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

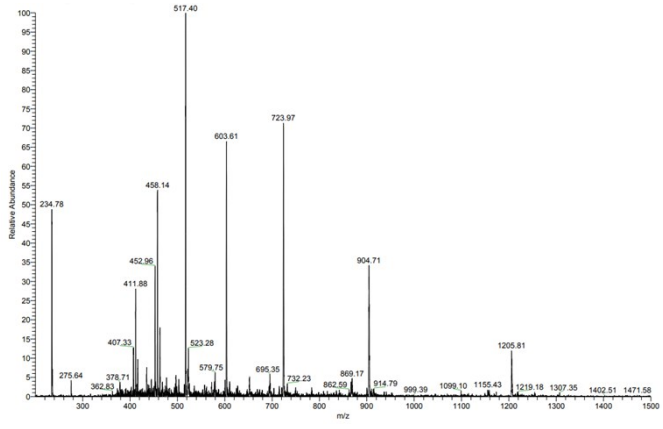

Fig. S10: ESI-MS of FIT-PNA **5**. M_{calc} = 3618.77, M_{obs} =3614.8.

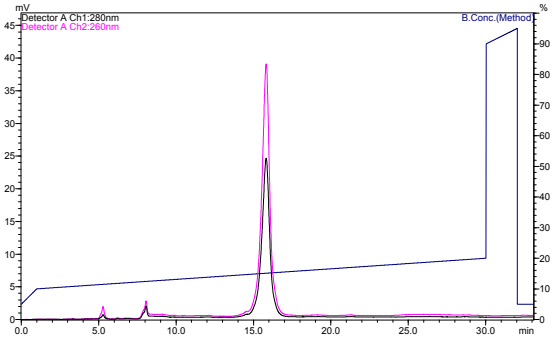

Fig. S11: HPLC chromatogram of FIT-PNA **6**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

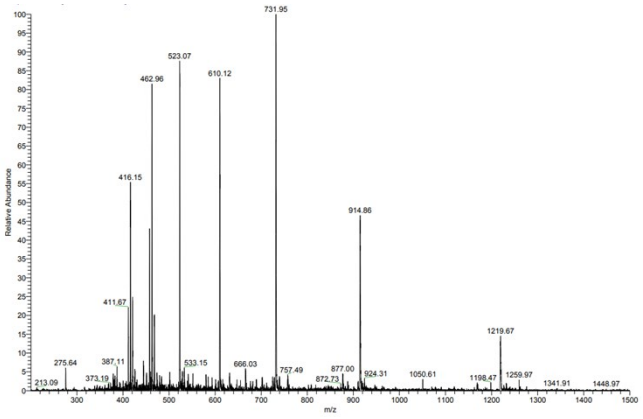

Fig. S12: ESI-MS of FIT-PNA **6**. M_{calc} = 3658.84, M_{obs} =3654.7.

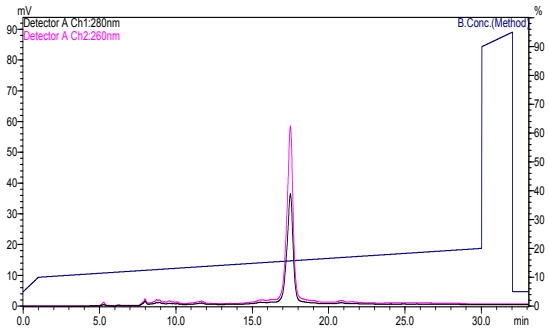

Fig. S13: HPLC chromatogram of FIT-PNA **7**. Eluents: A (0.1% TFA in water) and B (MeCN) were used in a linear gradient (10-15 % B in 30min) with a flow rate of 4mL/min.

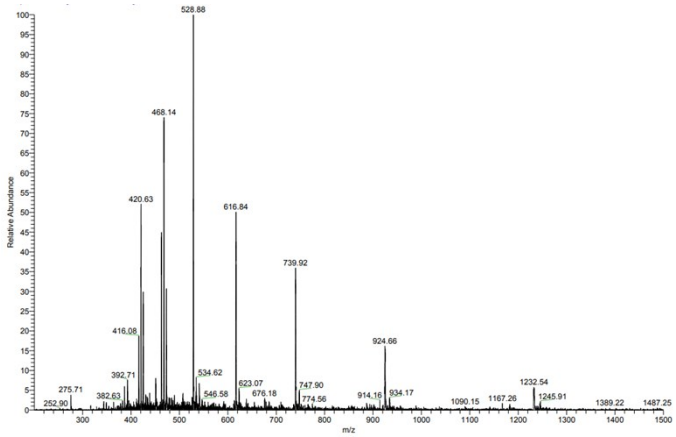

Fig. S14: ESI-MS of FIT-PNA **7**. M_{calc} = 3698.9, M_{obs} =3694.6.

Tm measurements of FIT-PNAs 1-7:

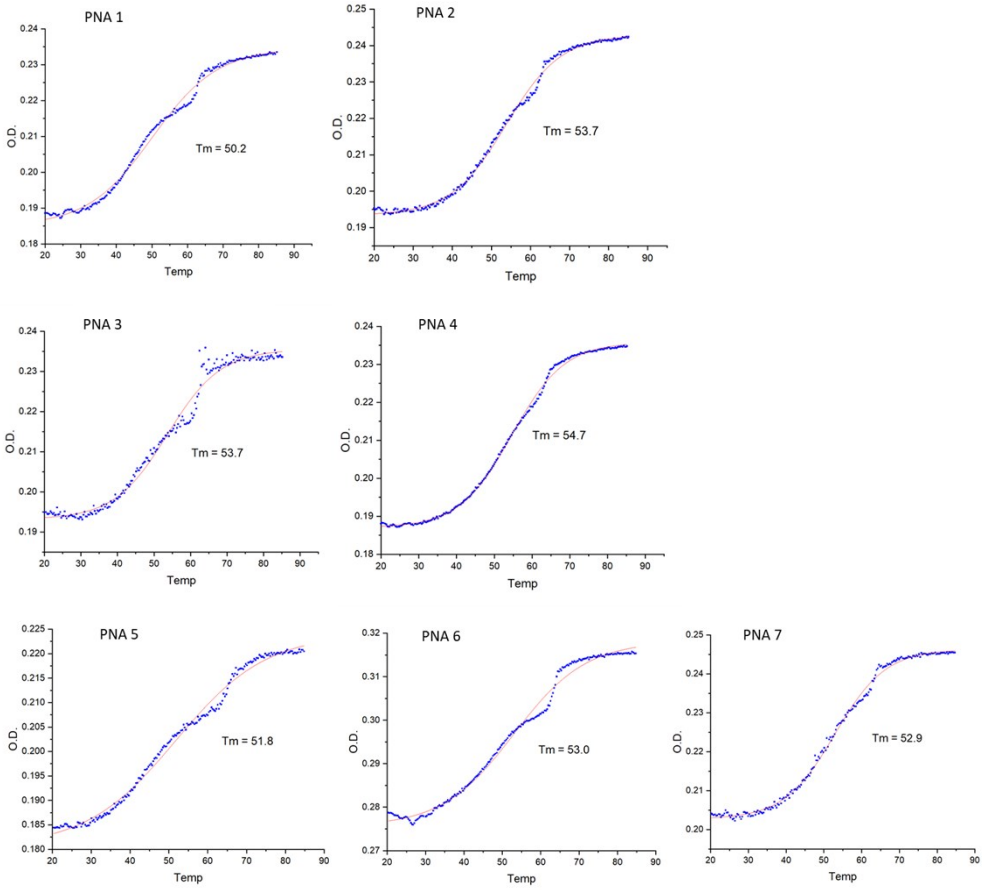

Fig. S15: Tm measurements of FIT-PNAs hybridized to complementary RNAs. All FIT-PNAs were annealed to complementary 11-mer RNAs (1:1 ratio) at 1μM for 2 hr at 37 °C in PBS. The OD was measured while heating the samples at 1°C / 1 min, from 20°C to 90° C.

Fluorescence spectra of mismatch sensitivity for single-modified cpFIT-PNAs (2, 3, and 6):

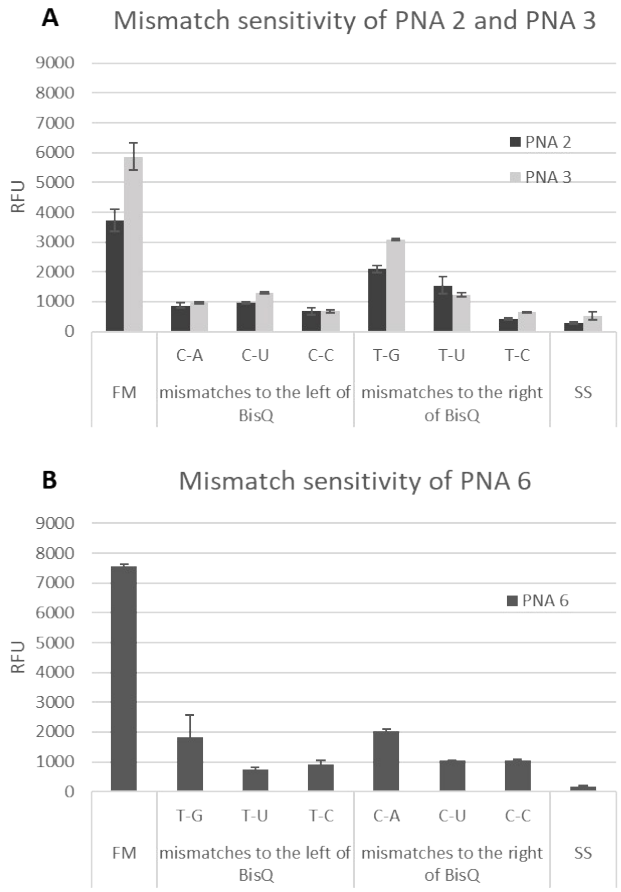

Fig. S16: Mismatch sensitivity of single substituted cpFIT-PNAs. **(A)** cpFIT-PNA 2 and 3 and **(B)** cpFIT-PNA 6. FIT-PNAs (0.5 μM) were annealed to all possible 11-mer RNAs (0.75 μM) and measured on a plate reader (λex=575 nm, λem= 615 nm, n=3).

Limit of detection

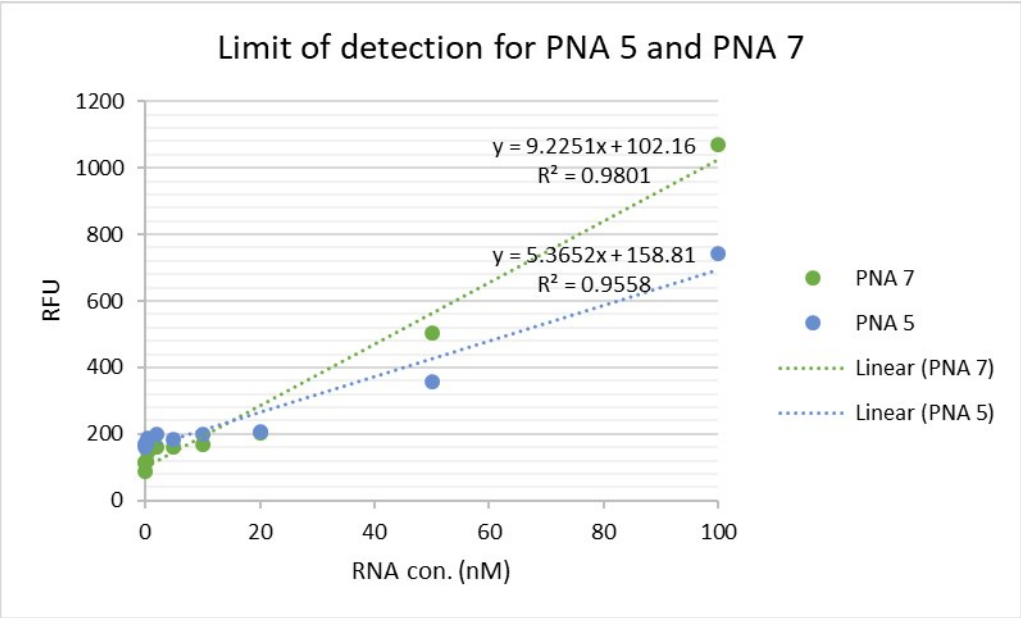

Fig. S17: LOD comparing cpFIT-PNA 7 to FIT-PNA 5. LOD was measured on a plate reader at constant PNA concentration of 0.5 μM adding different concentrations of complementary RNA. For annealing, the duplex solutions (on the plate) were incubated at RT overnight on a shaker (λex=580 nm, λem= 615 nm, n=5).

LOD was calculated by the equation: LOD=3.2*σ/slope. LOD for FIT-PNA 5 = 14.1 nM; LOD for cpFIT-PNA 7 = 1.8 nM.

References:

1. H. Zheng, M. Saha and D. H. Appella, *Organic Letters*, 2018, 20, 7637-7640
2. N. Kolevzon, D. Hashoul, S. Naik, A. Rubinstein and E. Yavin, *Chemical Communications*, 2016, 52, 2405-2407.
3. D. Magde, J. H. Brannon, T. L. Cremers and J. Olmsted, *The Journal of Physical Chemistry*, 1979, 83, 696-699.