Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

for

Clamping of RNA with PNA enables targeting of microRNA

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Contents

S1. Material and methods: synthesis of the PNA-conjugates.

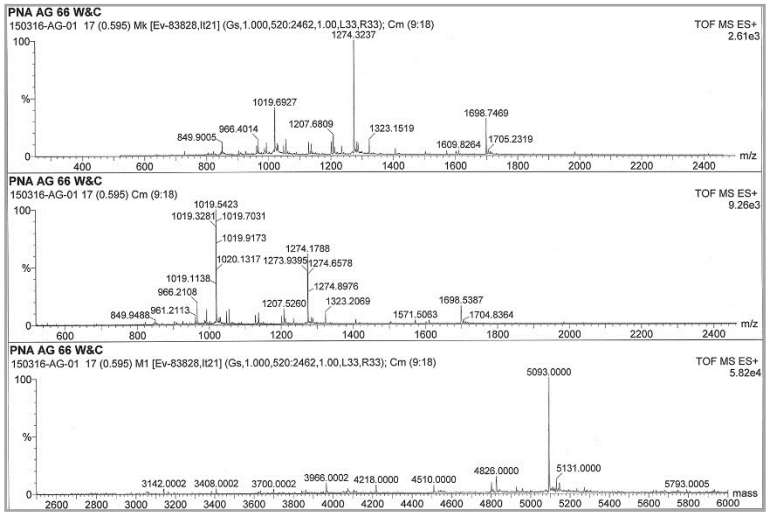
PNA building blocks were purchased from Link Technologies Ltd (Strathclyde, UK). Fmoc-αN-Lys(εN-Boc)OH, Fmoc-Dap(Mtt)OH as well as the building blocks for PEG linkers, 3-(2-(2-(9-Fluorenylmethyloxycarbonyl)aminoethoxy)ethoxy)propanoic acid (Fmoc-AEEP), 8-(9-Fluorenylmethyloxycarbonyl-amino)-3,6-dioxaoctanoic acid (Fmoc-AEEA) and 5-(9-Fluorenylmethyloxycarbonyl-amino)-3-oxapentanoic acid were from Iris Biotech Gmbh (Marktredwitz, Germany). PNA sequences were assembled automatically on a solid support (Rink Amide resin) using the manufacturer's protocol for the Astra Initiator Biotage peptide synthesizer with 9-fluorenylmethyloxycarbonyl (Fmoc)-chemistry and N,N'-diisopropylcarbodiimide (DIC) as coupling agent and ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) as additive. For BisPNA 6 (where the directection of the PNA sequence is reversed) Fmoc-Dap(Mtt)OH was first coupled to the Rink amide resin, then Lysine and the Watson-Crick binding sequence of the PNA was synthesized. After removal of the Mtt protection (as described in our synthesis of PNAzymes, Ghidini, A.; Murtola, M.; Strömberg, R *Org. Biomol. Chem.,* 2016, 14, 2768) the Dap was extended on the side-chain with the PEG-linkers and the triplex forming part of the PNA. High-resolution mass spectrometry (HRMS) was performed on a Micromass LCT electrospray time-of-flight (ES-TOF) mass spectrometer in acetonitrile–water 1:1 (v/v) solutions. The molecular weights of the oligoribonucleotide and peptide nucleic acid conjugates were reconstructed from the m/z values using the mass deconvolution program of the instrument (Mass Lynx software package). The RNA substrate was purchased HPLC purified from Thermoscientific. Concentrations of both RNA and PNA were determined by UV absorption at 260 nm and calculated from extinction coefficients obtained by the nearest neighbor approximation (Puglisi, J.D.; Tinoco Jr, I. *Methods in Enzymology* **1989**, 180, 304-325).

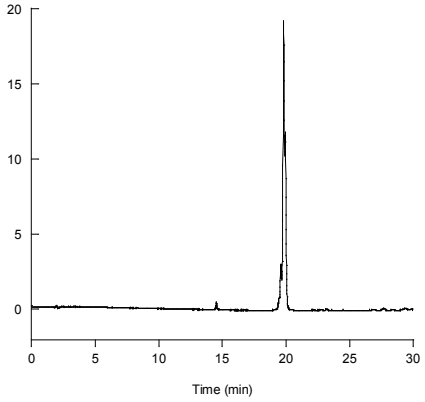
PNA 4 was purified with an Ascentis Express Supelco Peptide ES-C18 (2, 7 µm 150 × 4.6mm) column at 60 °C using a flow rate of 1mL/min and a linear gradient of 40% B for 30 min. (A) 0.1% TFA–aq., (B) 0.1% TFA–aq., 50 % MeCN. PNA 4, calculated mass, 2075; found ESI[M+], 2075.

All the other PNAs were purified with an Ascentis Express Supelco Peptide ES-C18 (2, 7 µm 150 × 4.6mm) column at 60 °C using a flow rate of 1mL/min and a linear gradient of 40% B for 27 min. (A) 0.1% TFA–aq., (B) 0.1% TFA–aq., 50 % MeCN. BisPNA 1, calculated mass, 5074; found ESI[M+], 5093. PNA 2, calculated mass, 3216; found ESI[M+], 3199. BisPNA 3, calculated mass, 5089; found ESI[M+], 5096. BisPNA 5, calculated mass, 5069; found ESI[M+], 5066. BisPNA 6, calculated mass, 5204; found ESI[M+], 5204. PNA 7, calculated mass, 3312; found ESI[M+], 3313. AntimiR 1, calculated mass, 7111; found ESI[M+], 7109. AntimiR 2, calculated mass, 7285; found ESI[M+], 7282. Ref PNA, calculated mass, 4442; found ESI[M+], 4442.

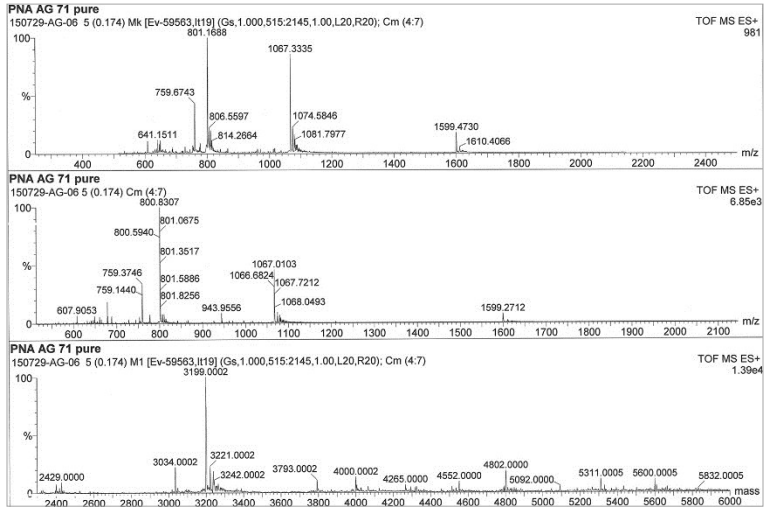
S2. Characterization of the PNA-conjugates: ESI-MS(+) (left panel) and chromatograms of purified PNA-conjugates (right panels).

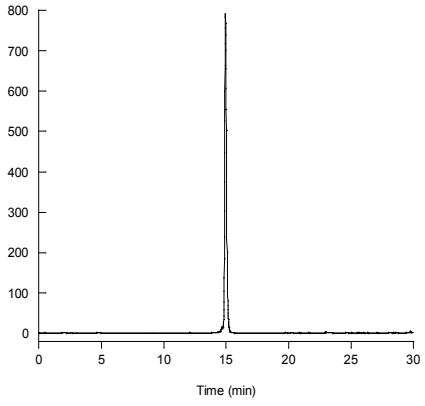
BisPNA 1



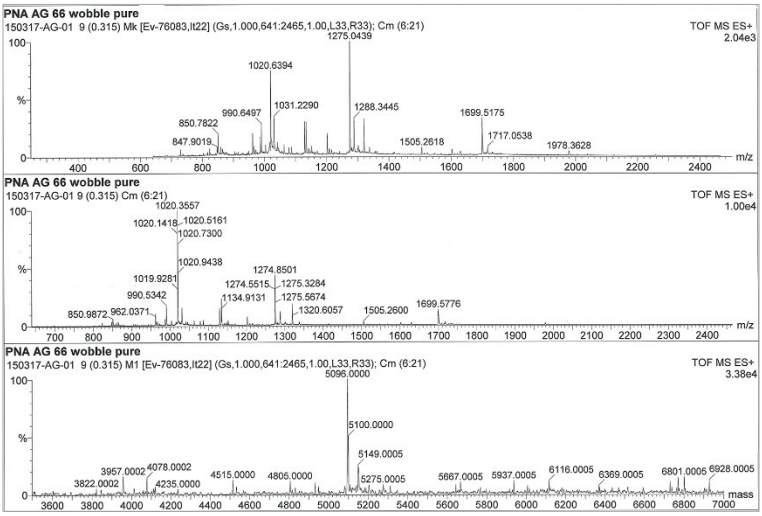


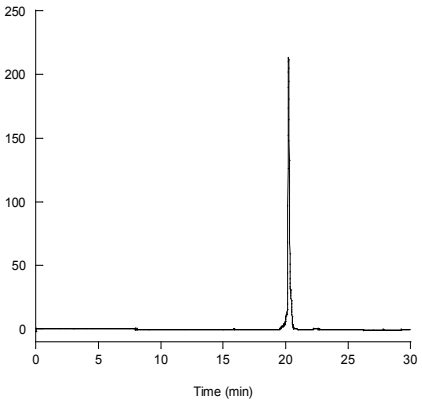
PNA 2



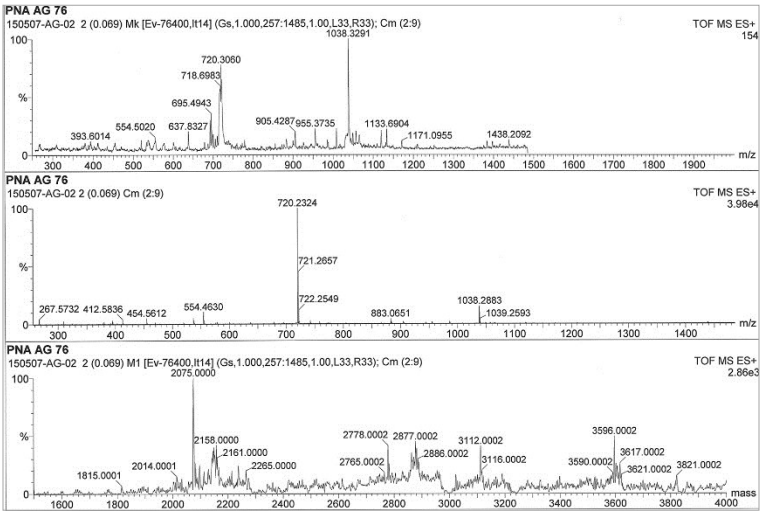


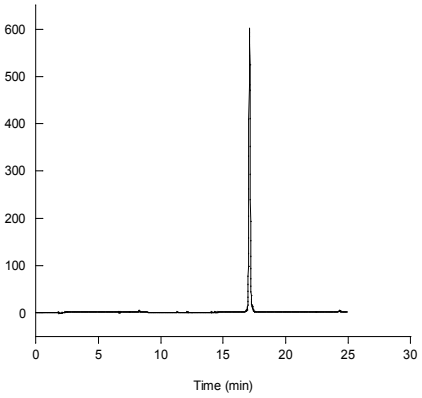
BisPNA 3



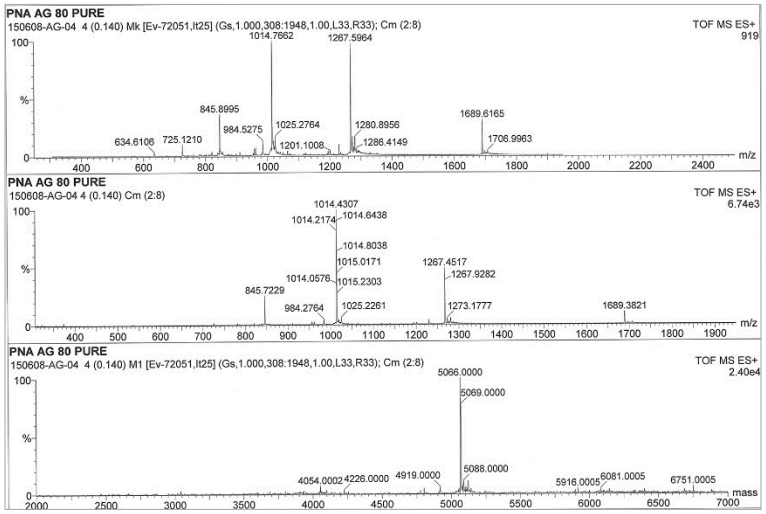


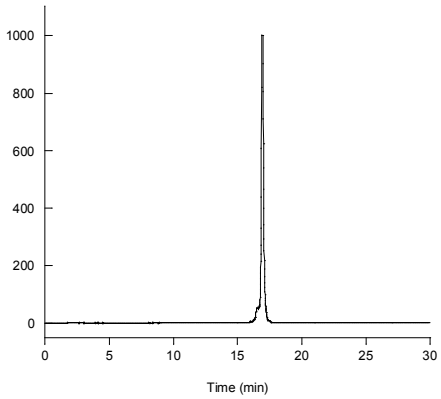
PNA 4



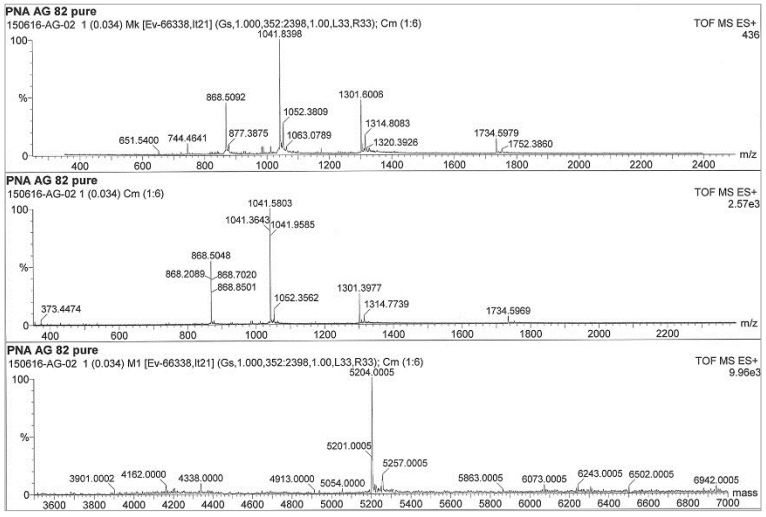


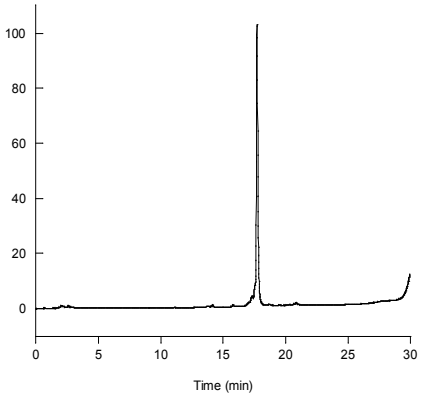
BisPNA 5



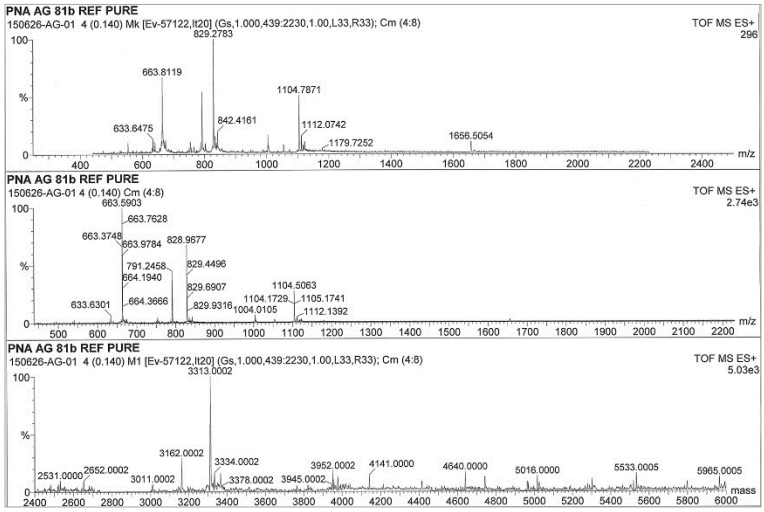


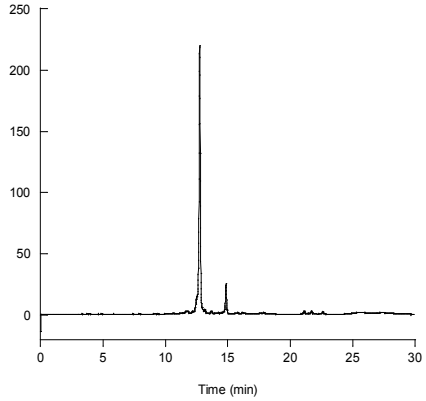
BisPNA 6



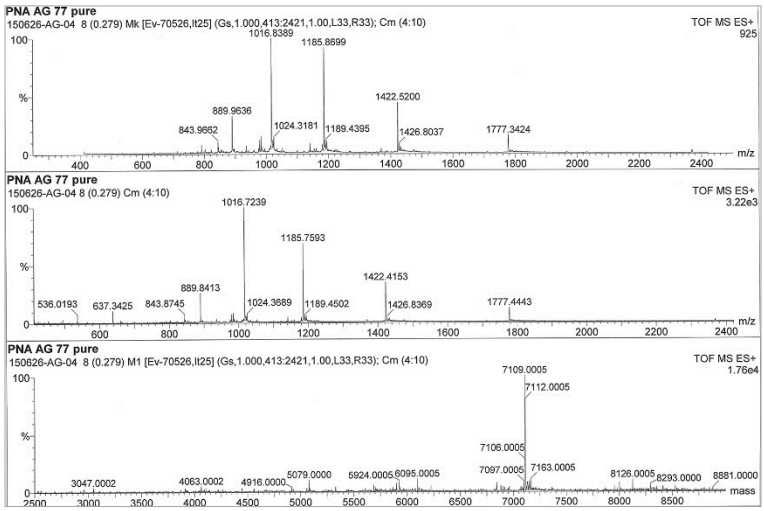


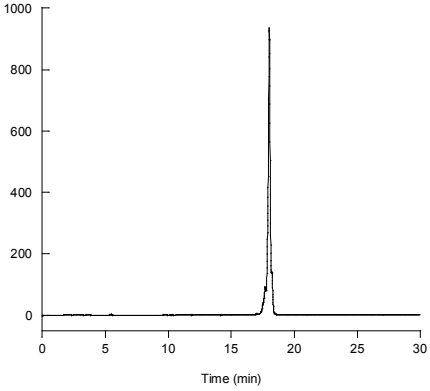
PNA 7





AntimiR 1





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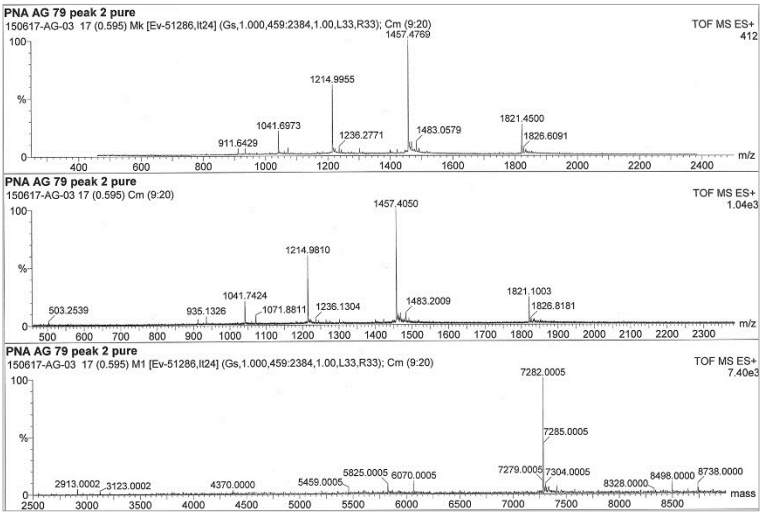
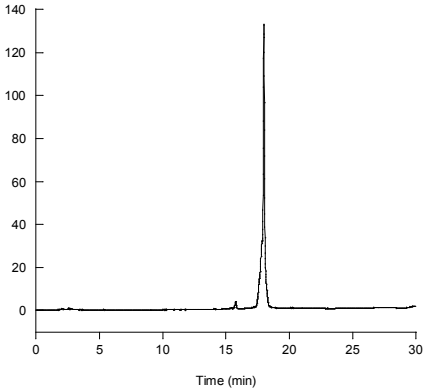
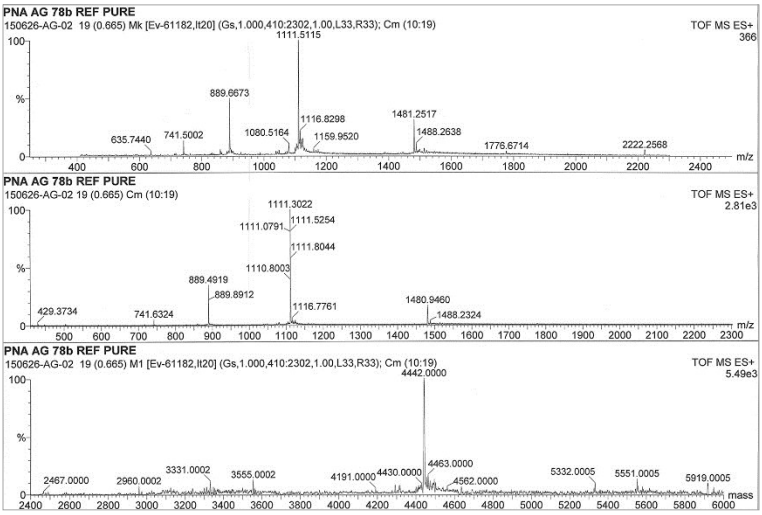
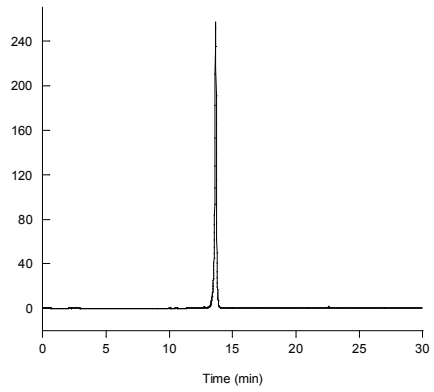




Ref PNA

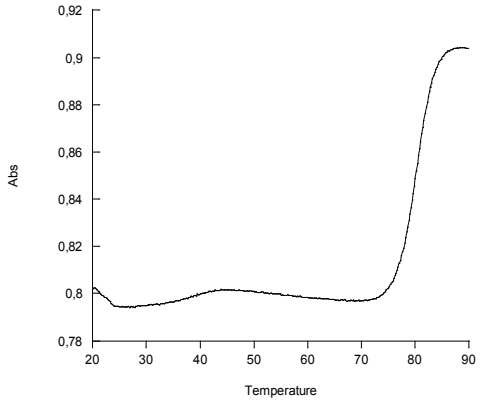


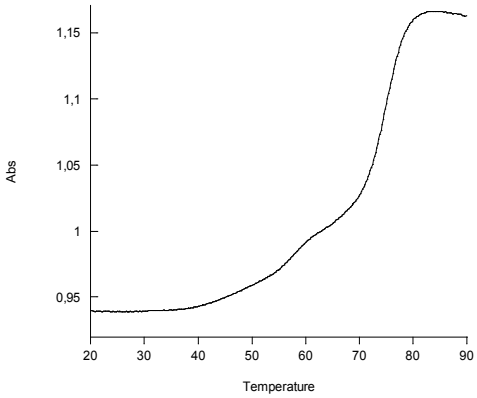


S3. Thermal Denaturation Analysis.

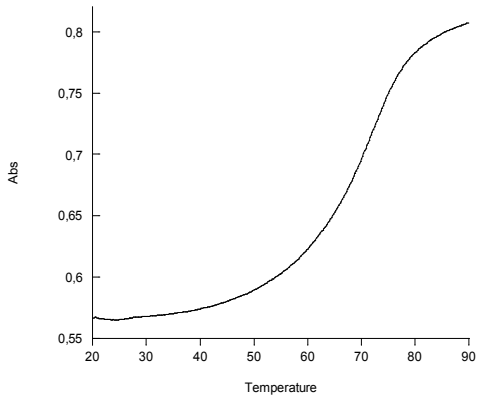
UV melting experiments, absorbance vs. temperature profiles, were measured on a Varian Cary 300 UV/VIS dual beam spectrophotometer (Varian) at 260 nm. Melting temperatures were measured with 1:1 molar mixtures of PNA and the corresponding target RNA (and in different ration in the case of PNA 4), each at a concentration of 4 µM, in a 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA at pH 7.0. The samples were heated to rapidly to 90 °C, left for 5 min and then allowed to cool to 20 °C. After equilibration for 10 min at the starting temperature, the dissociation was recorded by heating to 90°C at rate of 0.2°C min^{-1}. Varian Cary WinUV software version 3 was used to determine the melting temperatures (T_m).

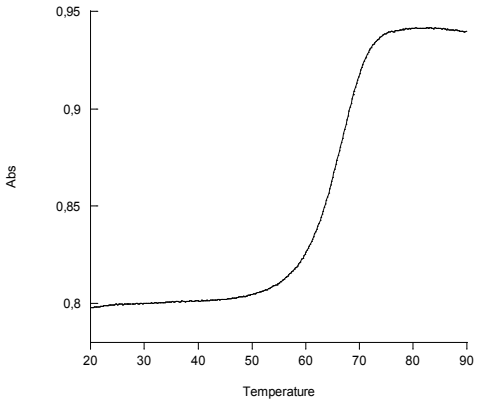
BisPNA 1 (Left panel - in presence of RNA and right panel - in presence of DNA)



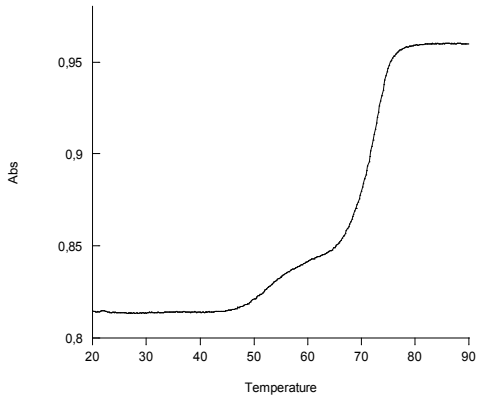


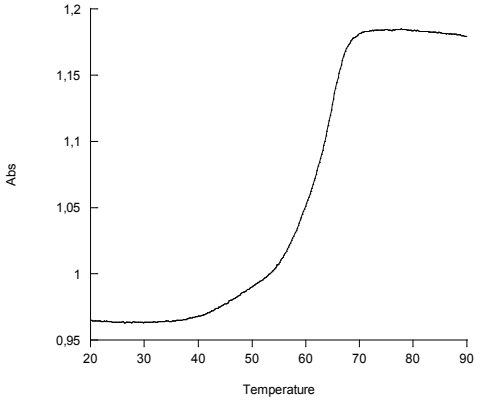
PNA 2 (Left panel - in presence of RNA and right panel - in presence of DNA)



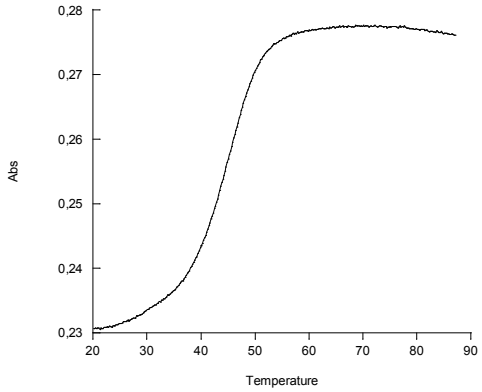


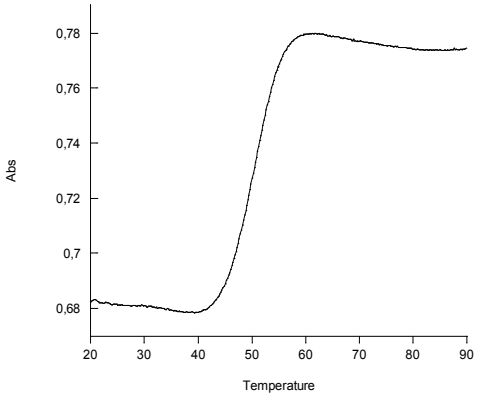
BisPNA 3 (Left panel - in presence of RNA and right panel - in presence of DNA)



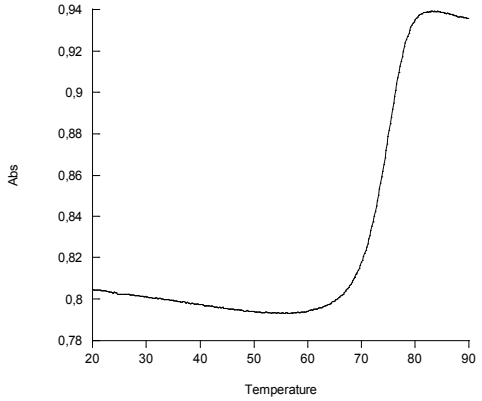


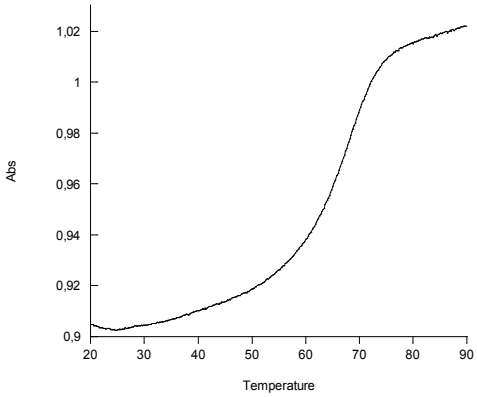
PNA 4 (Left panel – ratio PNA:RNA=1:1 and right panel - ratio PNA:RNA=3:1)



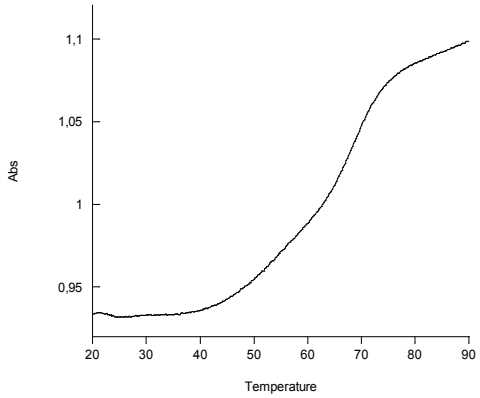


BisPNA 5 (left panel) and BisPNA 6 (right panel) in presence of RNA





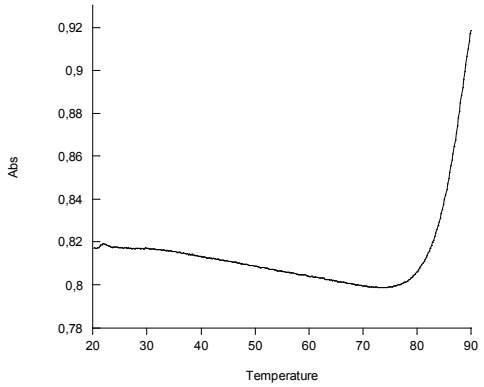
PNA 7 (in presence of RNA)



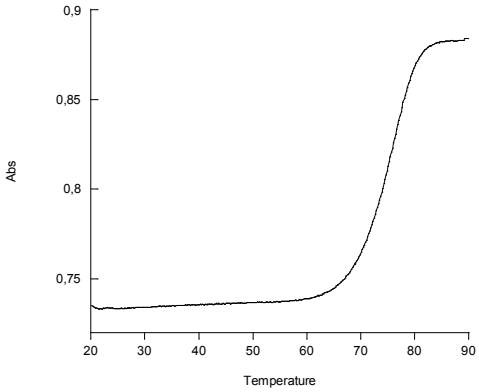
Melting run at pH 5.5

Buffer MES: 10 mM MES, 100 M NaCl, 100 mM EDTA

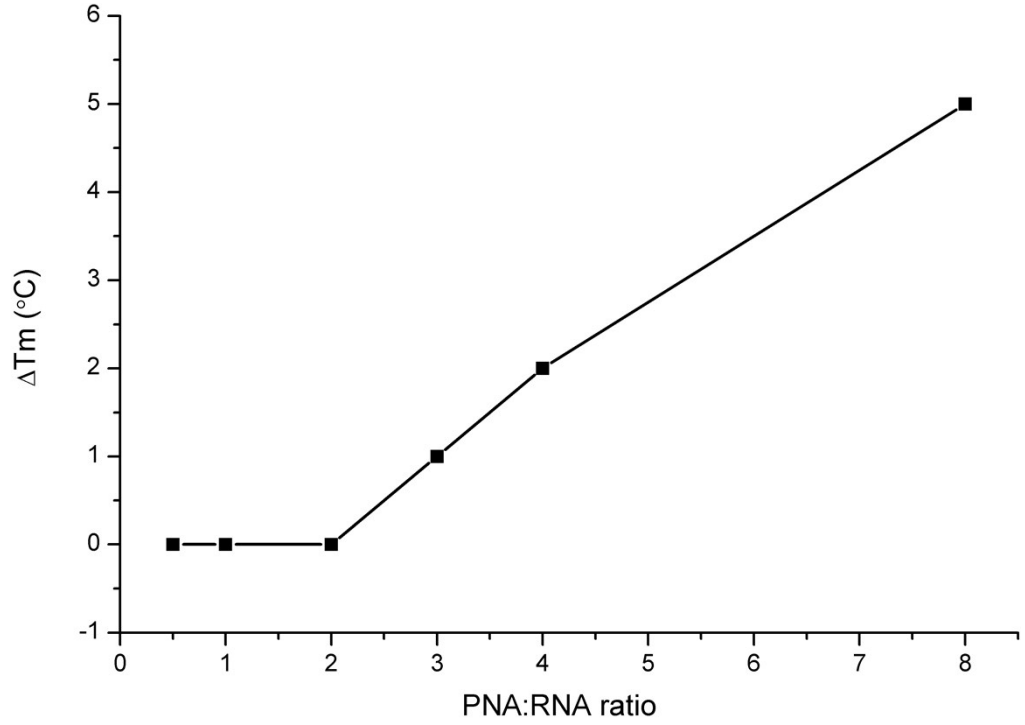
BisPNA 5 (in presence of RNA)



PNA 7 (in presence of RNA):



S4. Differences in melting point (ΔT_m) upon change of the ratio of **PNA 2** to **RNA target 1.** The experiments were performed as described for other T_m measurements with 4 µM RNA, but with 2, 4, 8, 12, 16 and 32 µM PNA.


6
5
4
3
2
1
0
-1
ΔTm (°C)
0
1
2
3
4
5
6
7
8
PNA:RNA ratio

S5. Circular Dichroism analysis.

The CD spectra were measured on an AVIV Circular Dichroism Spectrometer Model 410 with 1.0 cm path length cuvettes. The spectra were recorded as an average of five scans at 25°C and normalized by subtracting the background buffer scans. The CD spectra were obtained with 1:1 molar mixtures of PNA and the corresponding target RNA (and in different ration in the case of PNA 4), each at a concentration of 4 µM, in a 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA at pH 7.0. The samples were scanned between 230 nm and 380 nm.

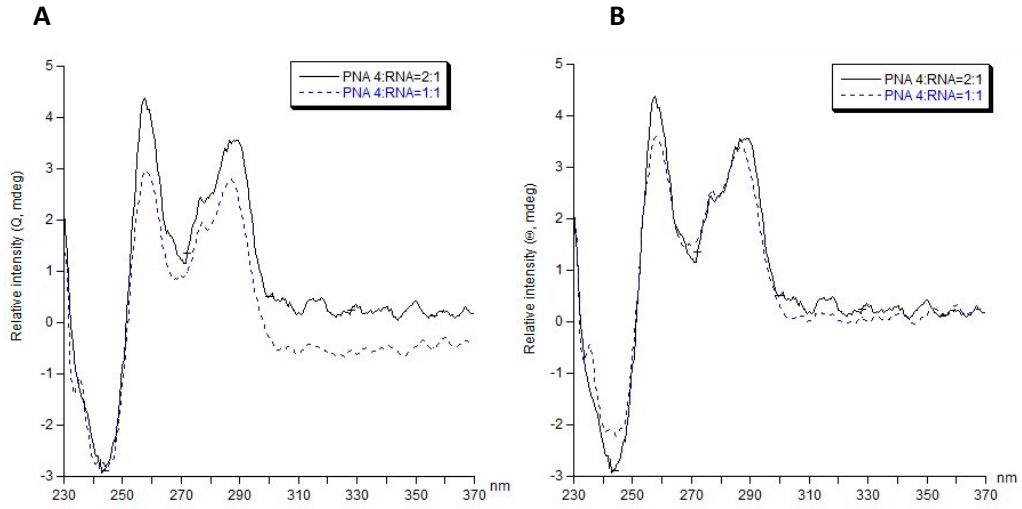


Figure S4a. CD spectra of PNA 4:RNA target 1 1:1 and 2:1 mixtures. A) as recorded. B) normalized (parallel shifted) by the average of differences at 366-370 nm.

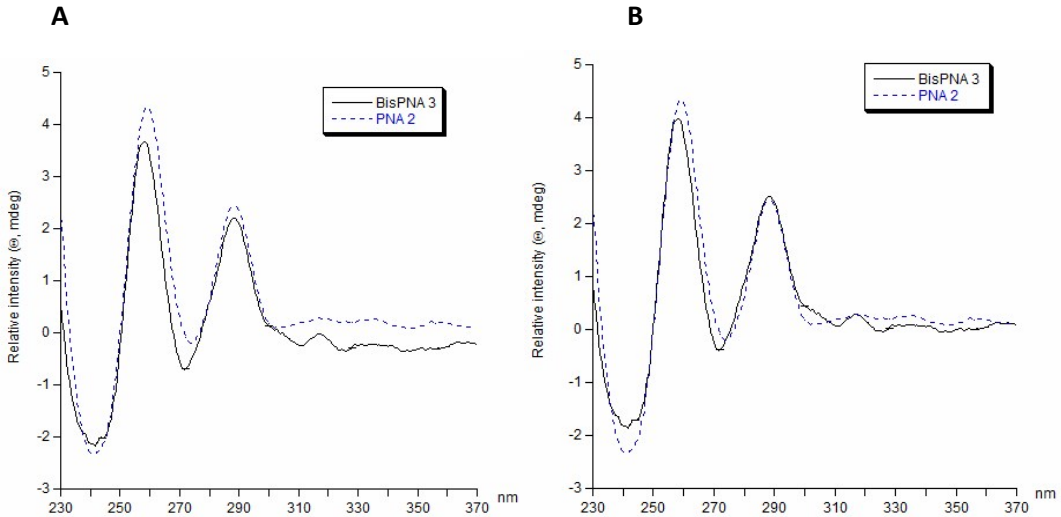


Figure S4b. CD spectra of RNA target 1 complexes with BisPNA 3 and PNA 2. A) as recorded. B) normalized (parallel shifted) by the average of differences at 366-370 nm.

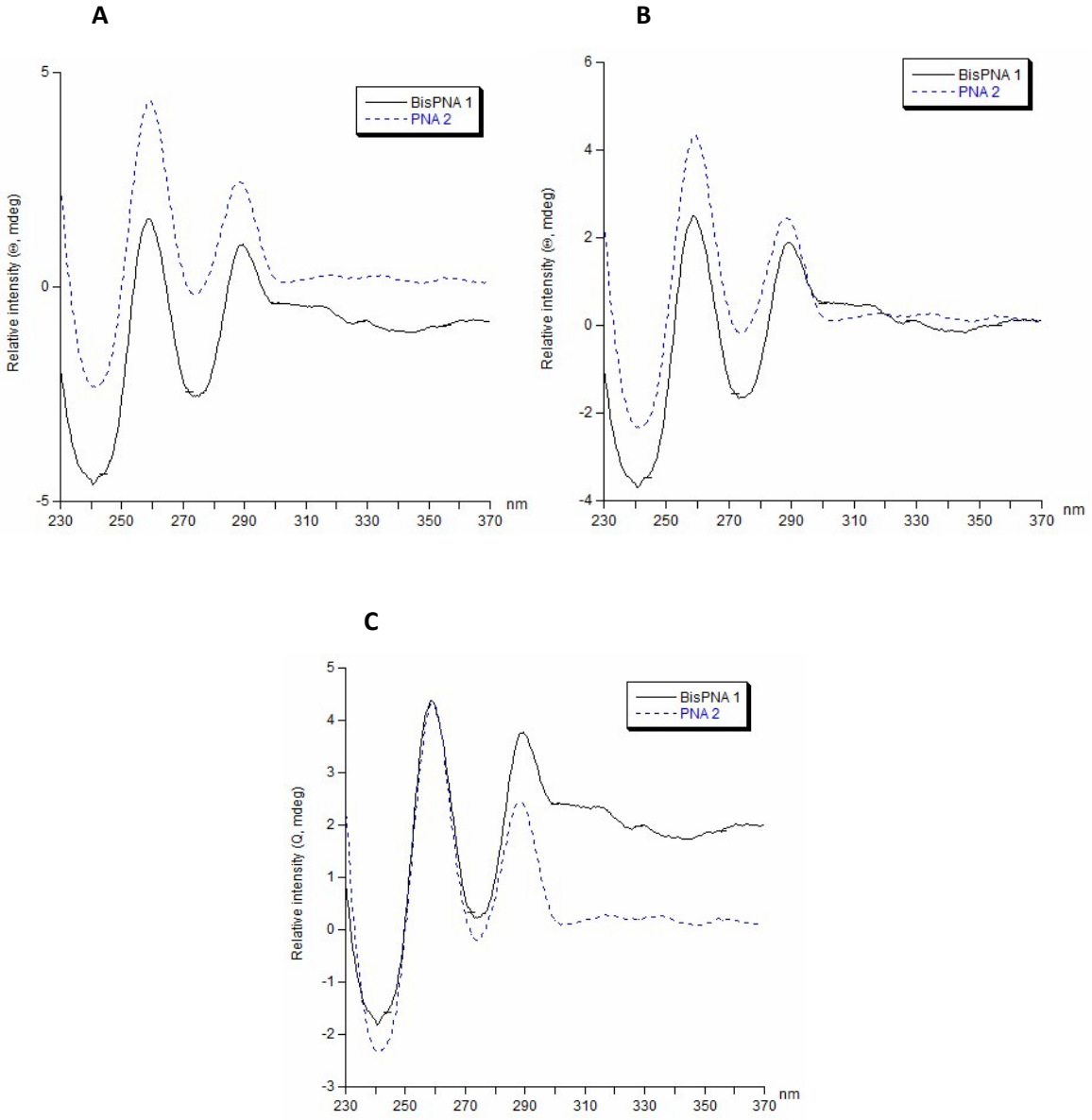


Figure S4c. CD spectra of RNA target 1 complexes with BisPNA 1 and PNA 2. A) as recorded. B) normalized (parallel shifted) by the average of differences at 366-370 nm. C) normalized (parallel shifted) by the difference at 260 nm.

S6. Gel shift experiments.

The miR-376b RNA was 5' end-labelled using [γ-^{32}P]ATP and T4 polynucleotide kinase (Fermentas) and purified using G-25 columns (GE Healthcare) according to the manufacturer`s protocol. 5nM of RNA was mixed with increasing concentration of PNA (AntimiR 1, AntimiR 2 and Ref PNA) in "pH 7.1" reaction buffer (20mM Tris-HCl, pH 7.1, 120mM KCl, 5mM NaCl, 0.5mM MgCl2, 10μM EDTA) or in "pH 5.5" reaction buffer (50mM Mes pH 5.5, 120mM KCl, 5mM NaCl, 0.5 mM MgCl2, 10μM EDTA) and incubated at 37 °C for 1, 24, 48 or 72 h. The samples were separated on 12% native polyacrylamide gel in 1X TBE buffer at room temperature for 3 h. Gels were exposed to a PhosphorImager screen (Fuji), scanned with Pharos FX™ Plus Molecular Imager (Bio-Rad) followed by ^{32}P -signal analysis using the Image One software (Bio-Rad).