

Supporting information

Influence of conjugation and other structural changes on the activity of Cu²⁺ based PNAzymes

Alice Ghidini^a, Merita Murtola^{a,b} and Roger Strömberg^{a,*}

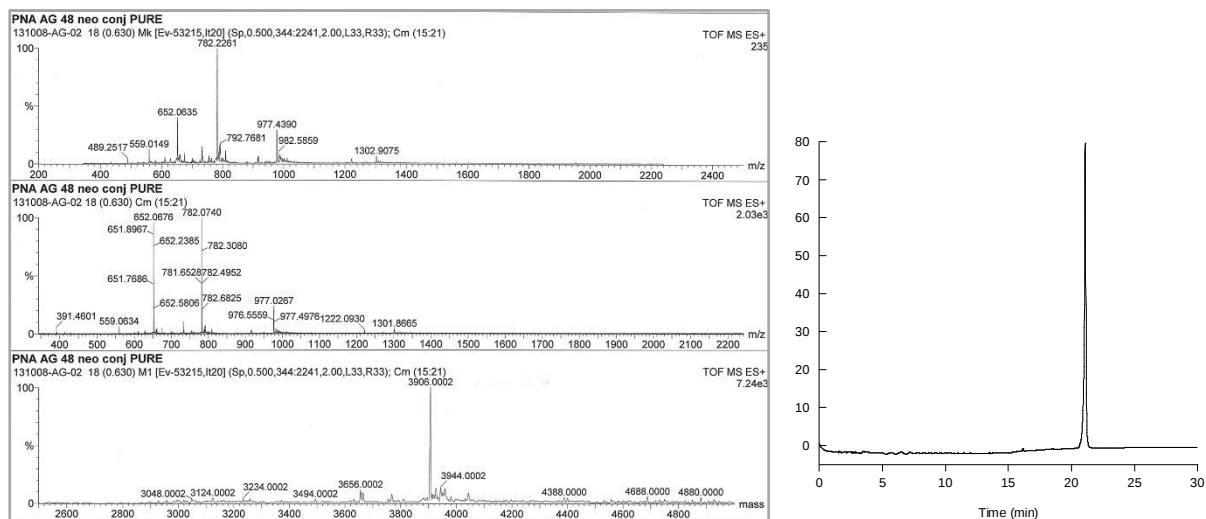
^aDepartment of Biosciences and Nutrition, Unit for Bioorganic Chemistry,
Karolinska Institutet, S-14183 Huddinge, Sweden

^bTurku University, Department of Chemistry, Turku 20014, Finland

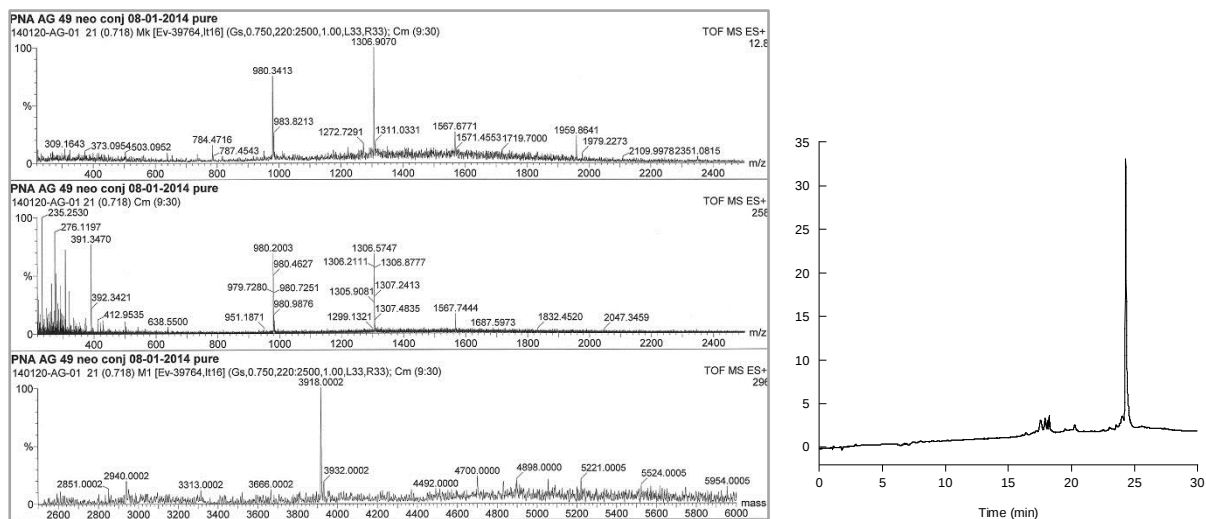
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S1. Characterization of the PNA-conjugates: ESI-MS(+) (left panel) and chromatograms of purified PNA-conjugates (right panels).

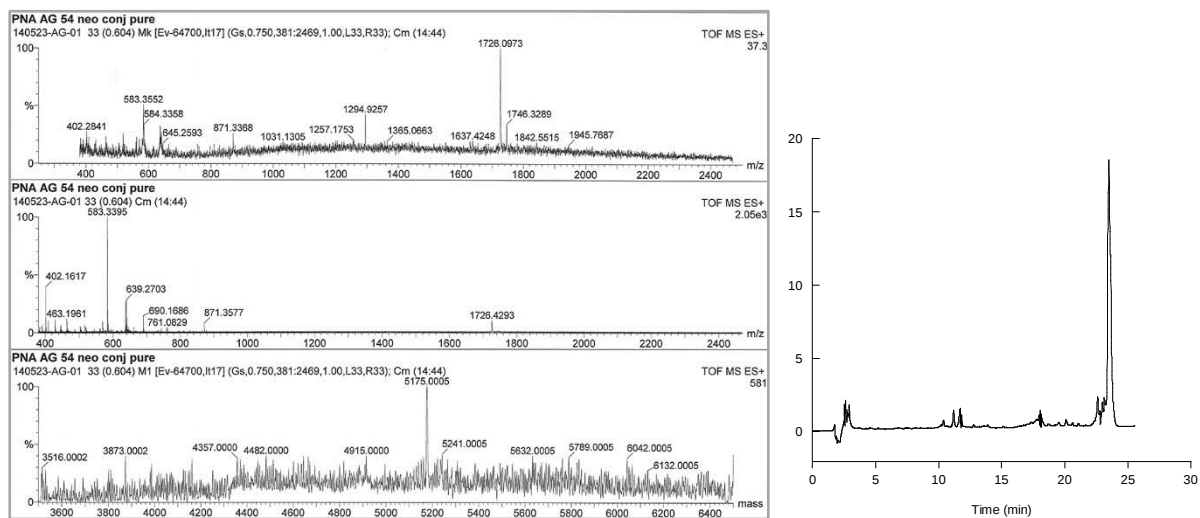
PNA 3



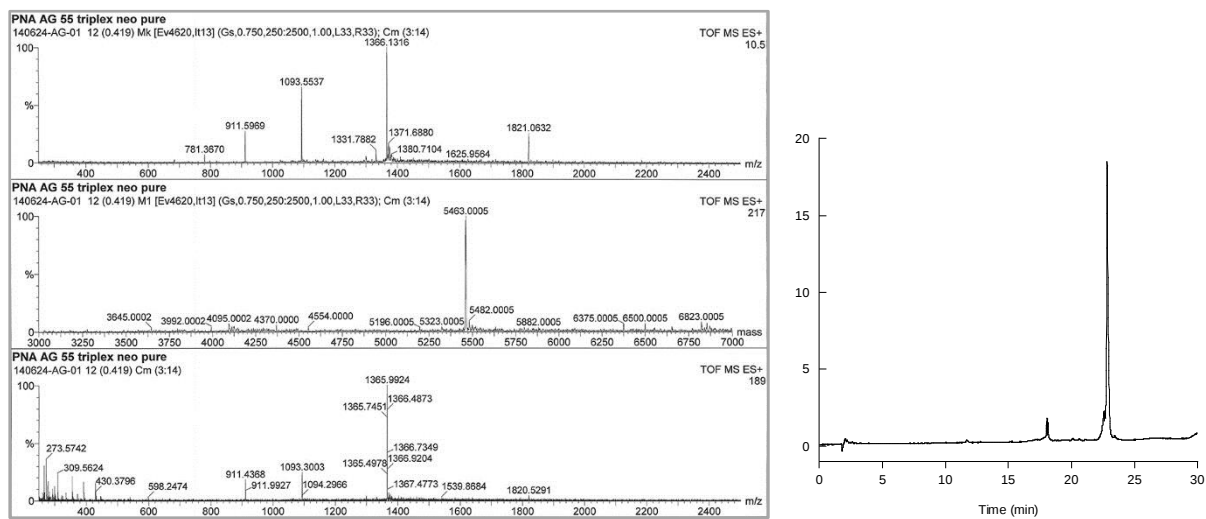
PNA 4



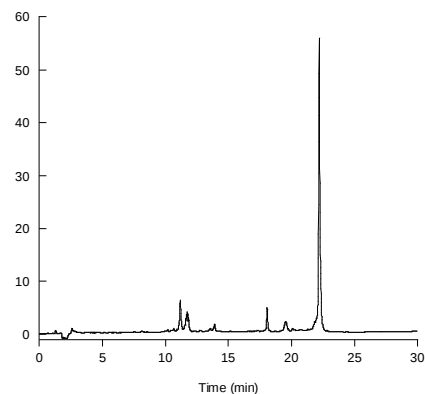
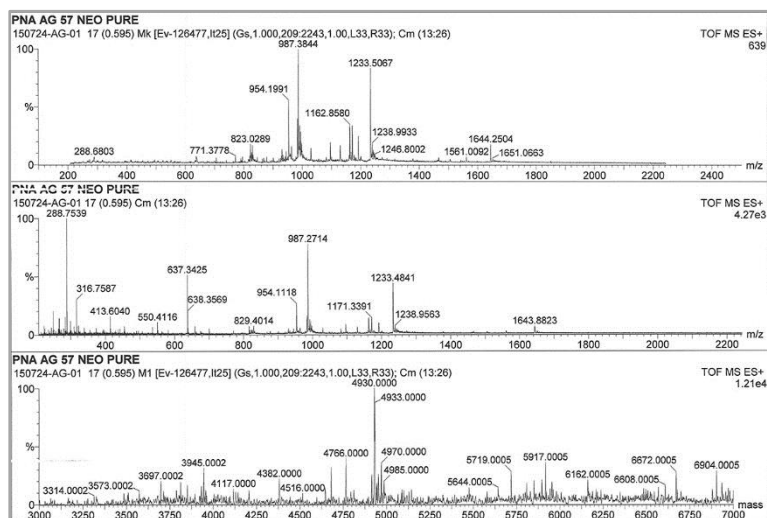
BisPNA 5



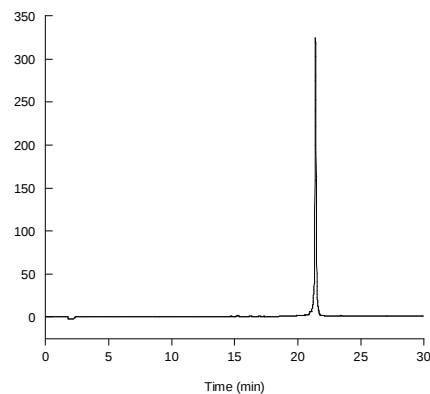
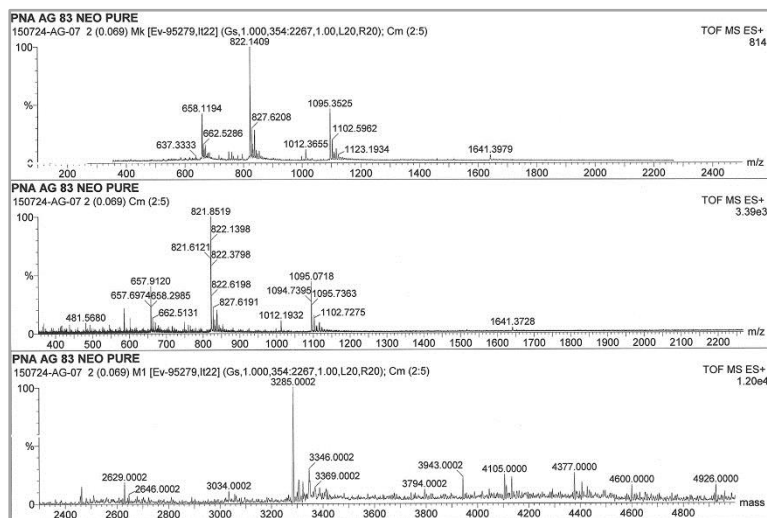
BisPNA 6



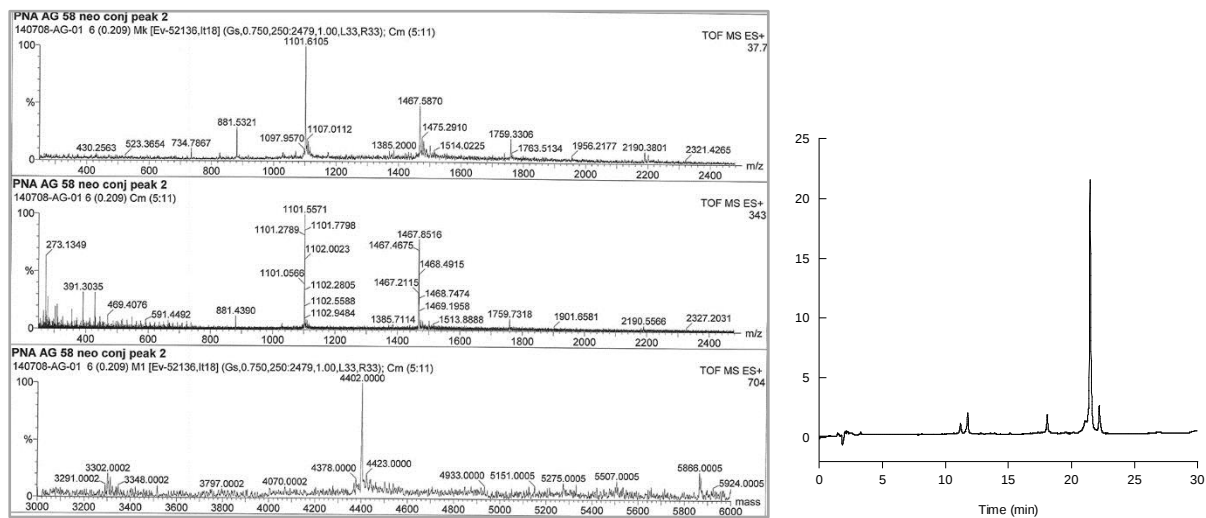
BisPNA 7



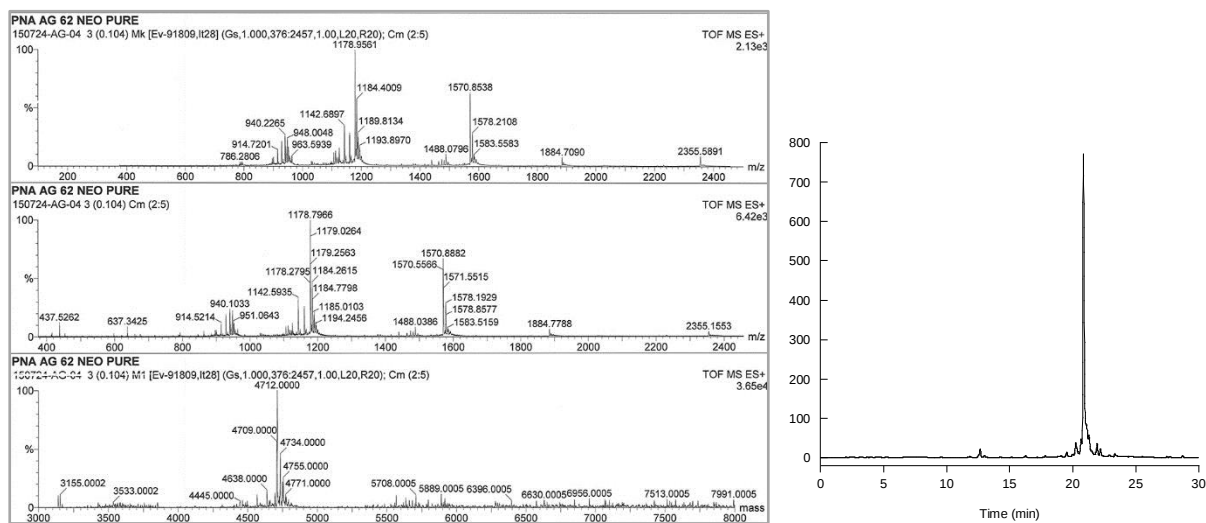
Ref PNA 7



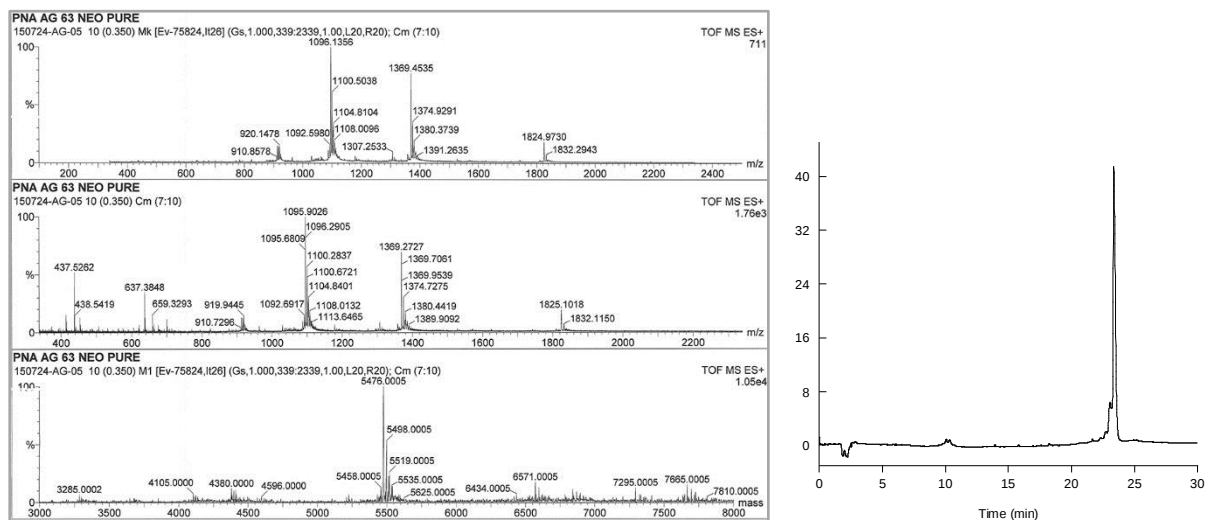
BisPNA 8



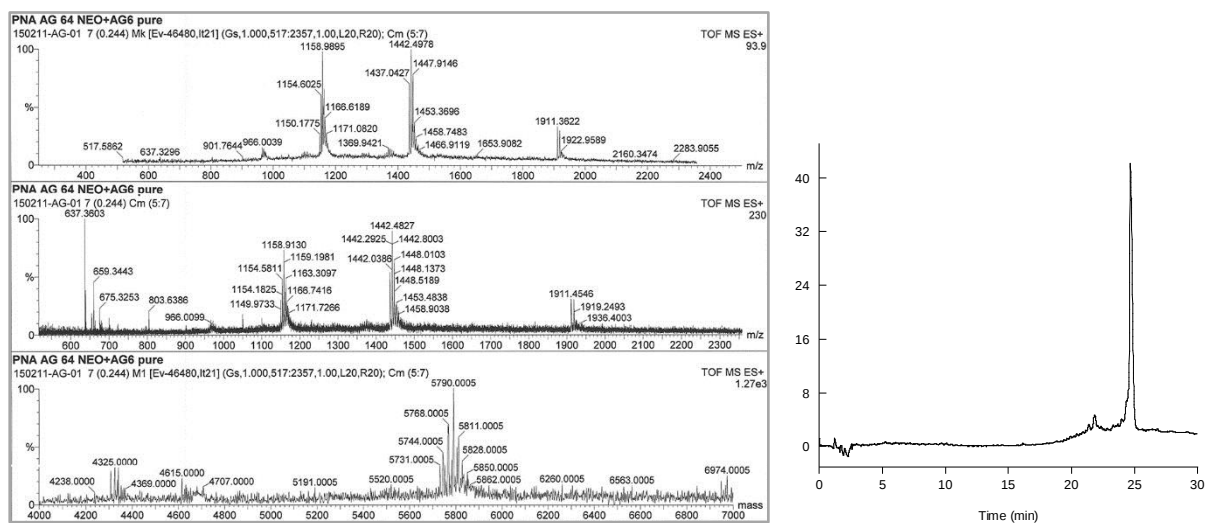
BisPNA 9



BisPNA 10



BisPNA 11



PNA AG 67 neo pure
150417-AG-01 13 (0.454) M1 [Ev-131676,124] (Gs,1.000,250,2500,1.00,L33,R33); Cm (8:16)
TOF MS ES+
1.54e3

PNA AG 67 neo pure
150417-AG-01 13 (0.454) Cm (8:16)
TOF MS ES+
8.11e3

PNA AG 67 neo pure
150417-AG-01 13 (0.454) M1 [Ev-131676,124] (Gs,1.000,250,2500,1.00,L33,R33); Cm (8:16)
TOF MS ES+
3.91e4

Time (min)

PNA AG 72 NEO PURE
150724-AG-06 3 (0.105) M1 [Ev-56566,1t23] (Gs,1,000,450:2270,1.00,L20,R20); Cm (2:5)
TOF MS ES+ 480

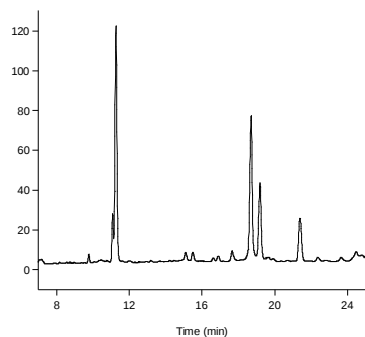
PNA AG 72 NEO PURE
150724-AG-06 3 (0.105) Cm (2:5)
TOF MS ES+ 1.15e3

PNA AG 12 NEO PURE
150724-AG-06 3 (0.105) M1 [Ev-56566,1t23] (Gs,1,000,450:2270,1.00,L20,R20); Cm (2:5)
TOF MS ES+ 5.77e3

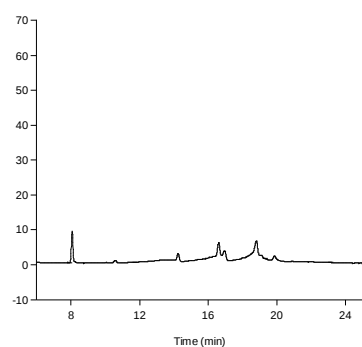
Time (min)

S2. Example of IE-HPLC chromatograms from kinetic studies of cleavage of the respective RNAs by BisRNA conjugates 5-12.

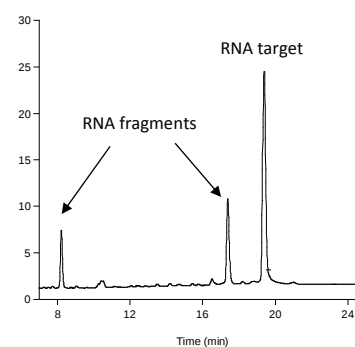
BisPNA 5 (after 80 min incubation)



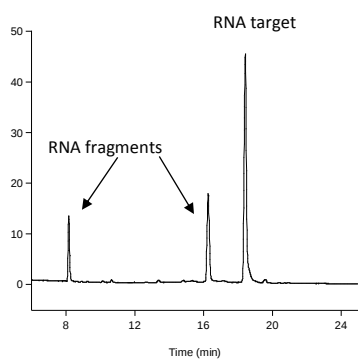
RNA target 2 with BisPNA 6 (after 80 min incubation)



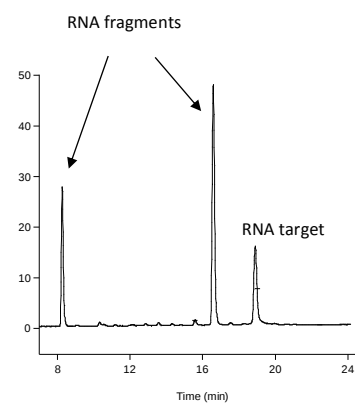
Turnover experiment:
(after 36 h incubation)
PNA : RNA = 1 : 5
PNA (0,8 μ M) and RNA (4 μ M)



RNA target 4 with BisPNA 7 (after 30 min incubation)

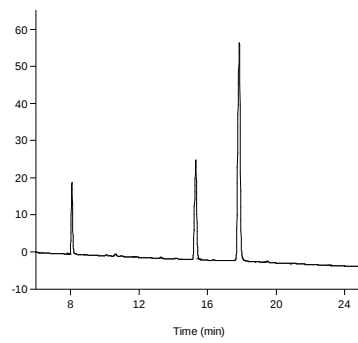


Turnover experiment:
(after 36 h incubation)
PNA : RNA = 1 : 5
PNA (0,8 μ M) and RNA (4 μ M)

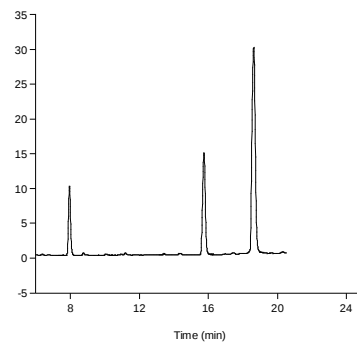


RNA target 5 with:

BisPNA 8 (after 30 min incubation)

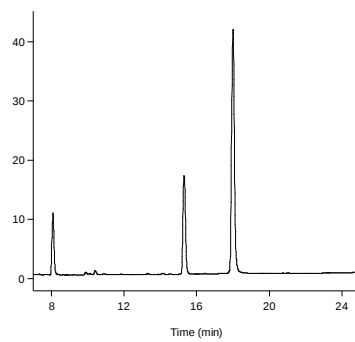


BisPNA 9 (after 30 min incubation)



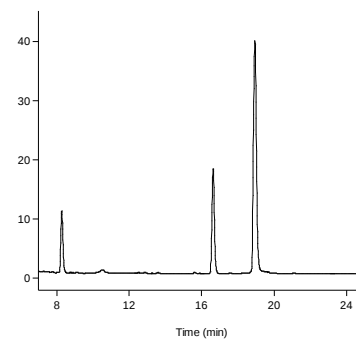
RNA target 5 with BisPNA 10

(after 30 min incubation)



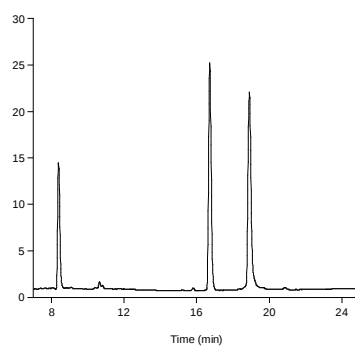
RNA target 4 with BisPNA 11

(after 30 min incubation)

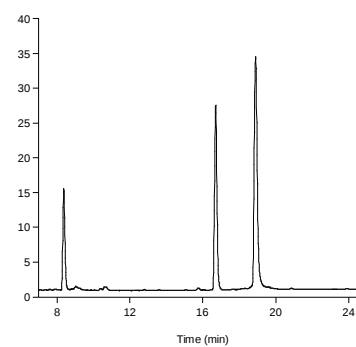


RNA target 6 with:

BisPNA 12 (after 30 min incubation)



BisPNA 13 (after 30 min incubation)

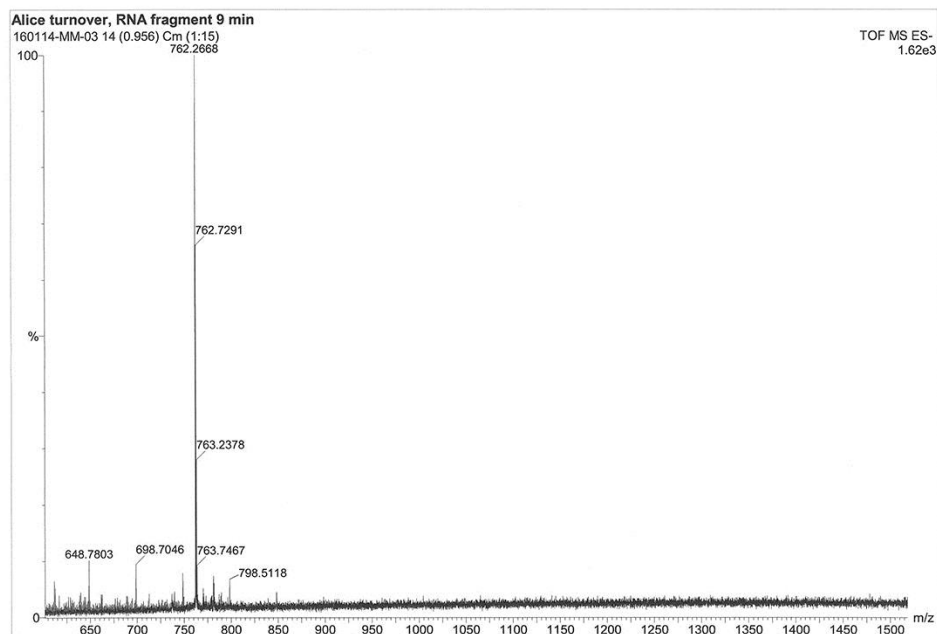


S3. Table S1. Cleavage rates with PNA 3,4,7 and BisPNA 7-13 and substrate half lives of the corresponding RNA targets.

PNA and BisPNA cleaver	k (min⁻¹)	t_{1/2} (h)	t_{1/2} (min)
PNA 3	0,0071	1,62	97
PNA 4	0,0037	3,14	188
BisPNA 7	0,014	0,86	51
PNA 7	0,0024	4,85	291
BisPNA 8	0,016	0,72	43
BisPNA 9	0,017	0,68	41
BisPNA 10	0,015	0,76	45
BisPNA 11	0,016	0,75	45
BisPNA 12	0,022	0,53	32
BisPNA 13	0,024	0,48	29

S4: Mass spectra of fragments formed upon cleavage of RNA target 5 with BisPNA 13. Fragment 1 = 9 min retention time and Fragment 2 = 17 min retention time in the IE-HPLC chromatograms in Figure 5.

Fragment 1 (m/z with $z = -2$):



Fragment 2 (m/z with $z = -3$)

