

*Supplementary Information to*

**Further Probing of Cu<sup>2+</sup>-Dependent PNAzymes Acting as Artificial RNA Restriction Enzymes**

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Representative chromatograms of the following HPLC analyses are included (time points at which the aliquots were collected indicated in parentheses):

**S1** Cleavage of 3-nucleotide bulge-forming RNAs **1-15** by PNAzyme **I** (24 h)

**S2** ESI MS spectra of fragments (2',3'-cyclic phosphates) produced in the cleavage of RNA **3** and **6**

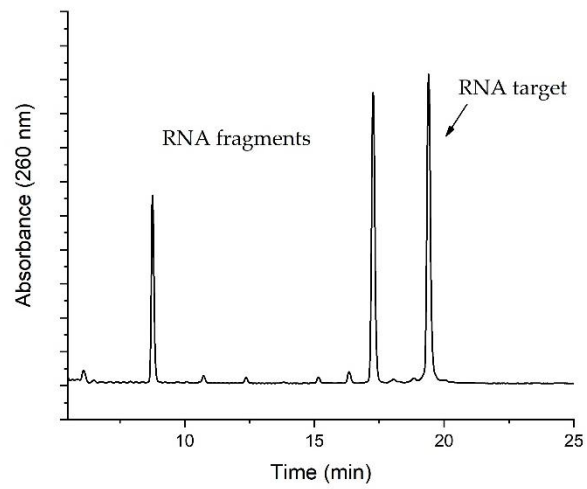
**S3** Cleavage of (or the lack thereof) modified 4-nucleotide bulge-forming RNAs **16** (1 h) and **17-19** by PNAzyme **I** (24 h) and RNA **16** in the presence of PNA-phenanthrene conjugate (**Y**) (24 h)

**S4** Cleavage of 4-nucleotide bulge-forming RNAs **16, 20-24** (30 min) promoted by PNAzyme **I** or **II**

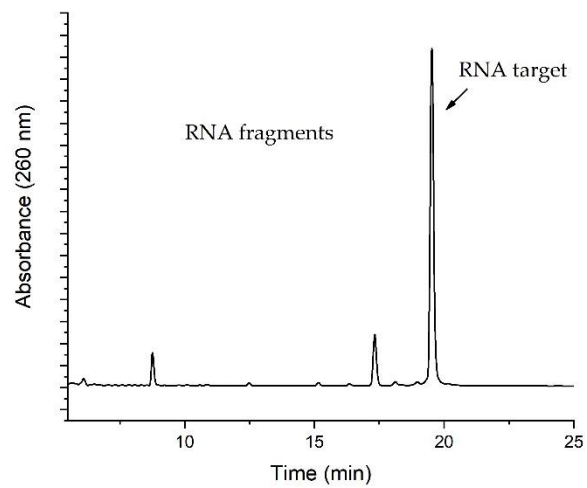
**S5** RP HPLC analysis of PNAzyme **II**

S1. Cleavage of 3-nucleotide RNA bulge-forming RNAs 1-15 by PNAzyme I (24 h)

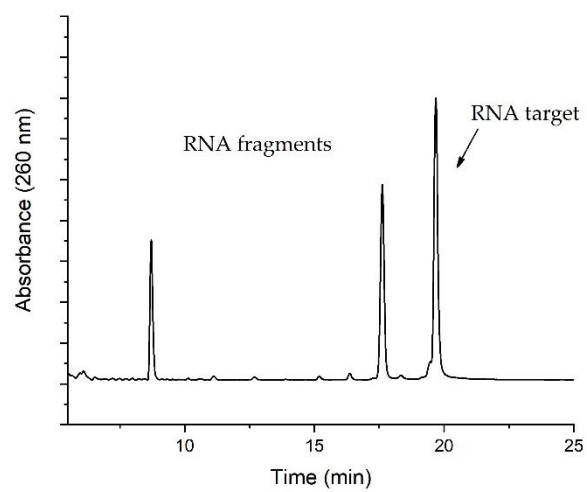
RNA 1 (5'-AAA-3')



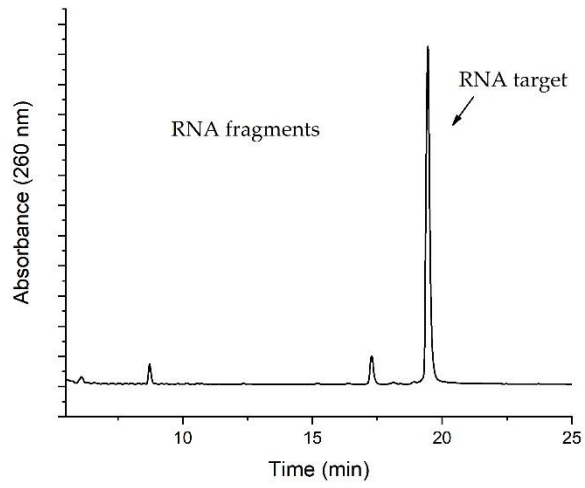
RNA 2 (5'-AUA-3')



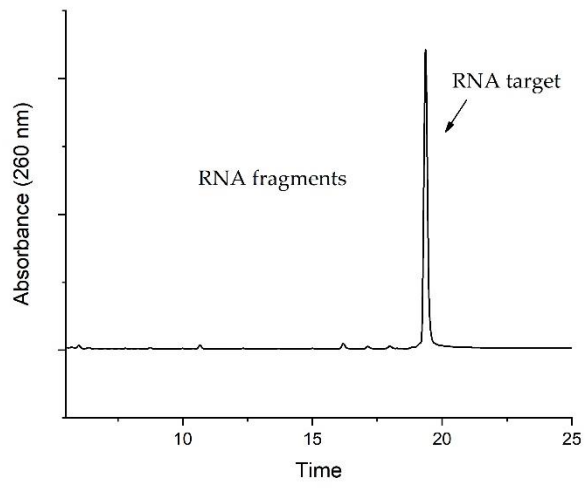
RNA 3 (5'-AGA-3')



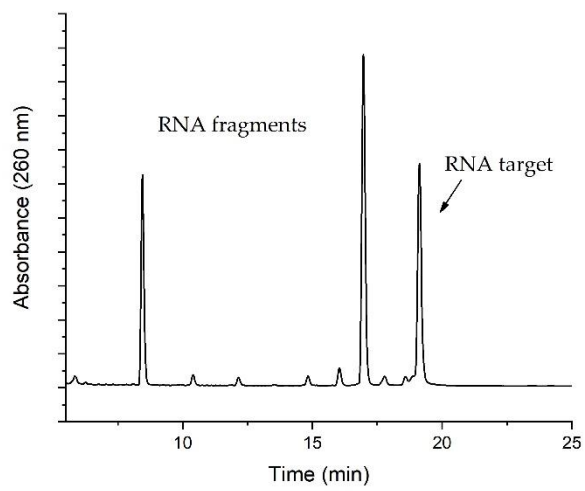
RNA 4 (5'-ACA-3')



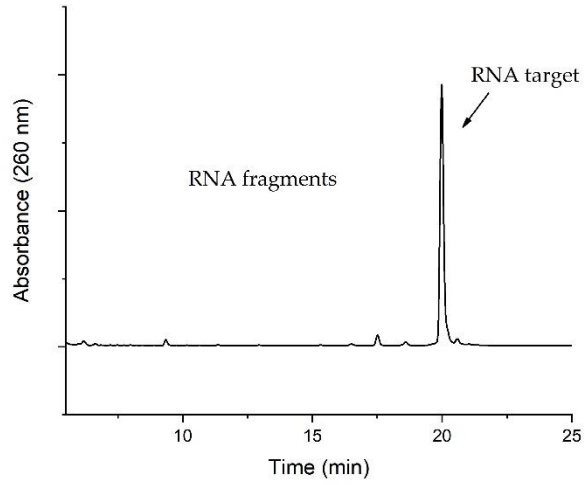
RNA 5 (5'-AAU-3')



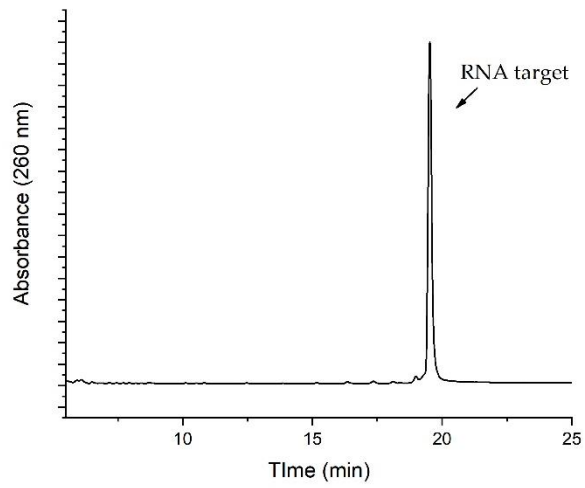
RNA 6 (5'-UAA-3')



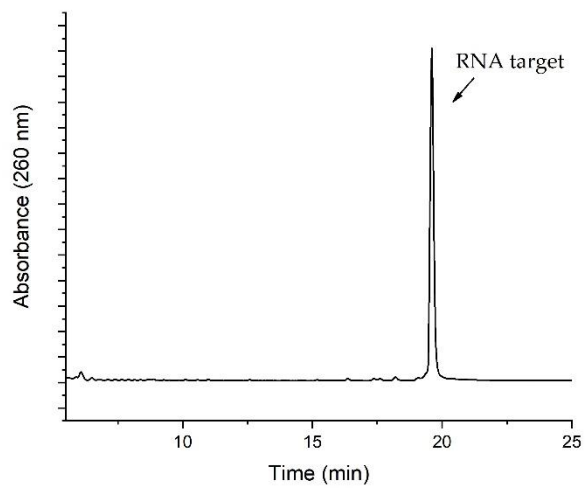
RNA 7 (5'-AUG-3')



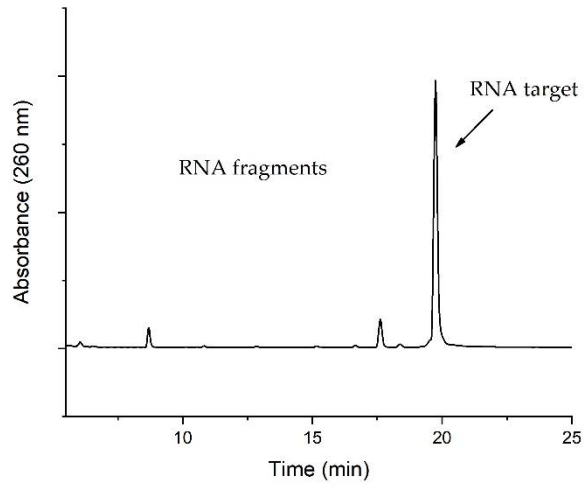
RNA 8 (5'-AUC-3')



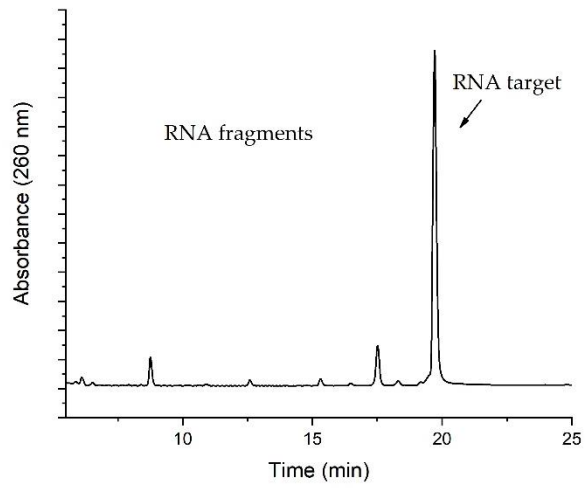
RNA 9 (5'-AUU-3')



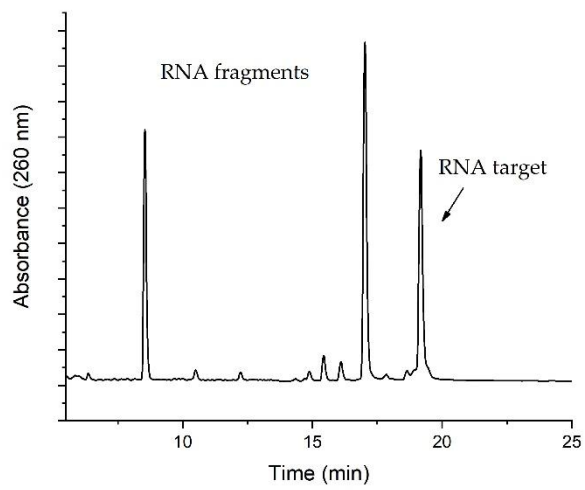
RNA 10 (5'-GUA-3')



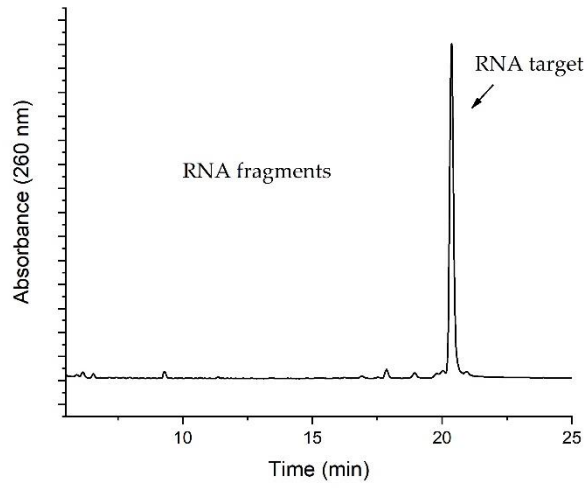
RNA 11 (5'-CUA-3')



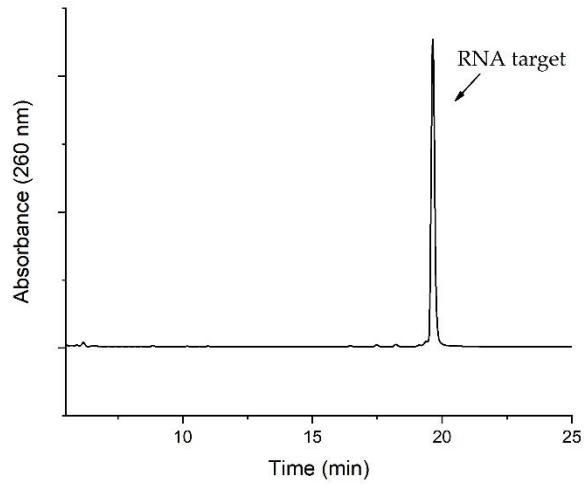
RNA 12 (5'-UUA-3')



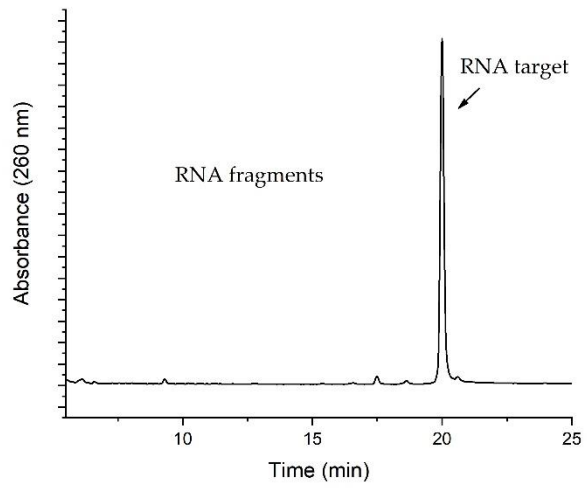
RNA 13 (5'-GUG-3')



RNA 14 (5'-CUC-3')



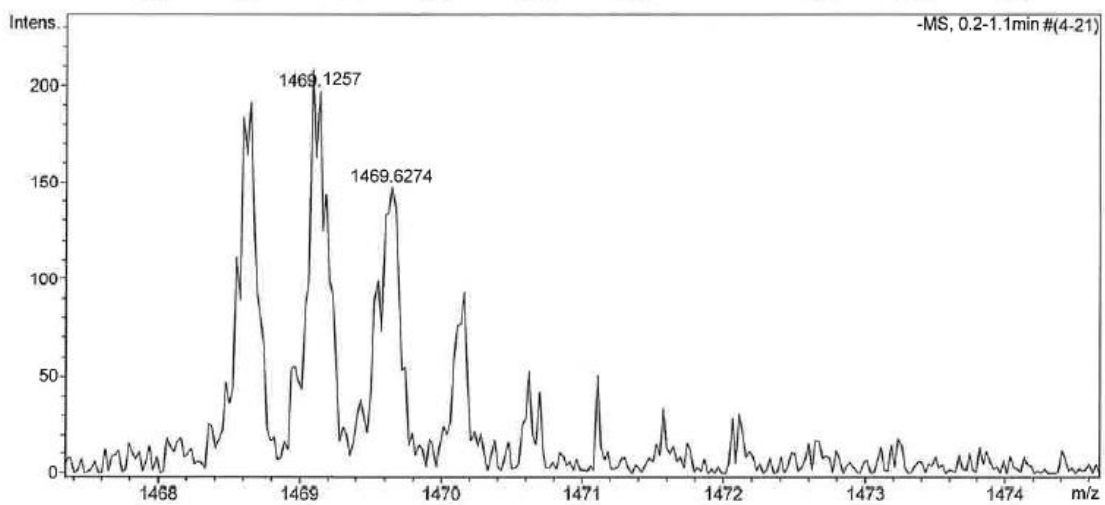
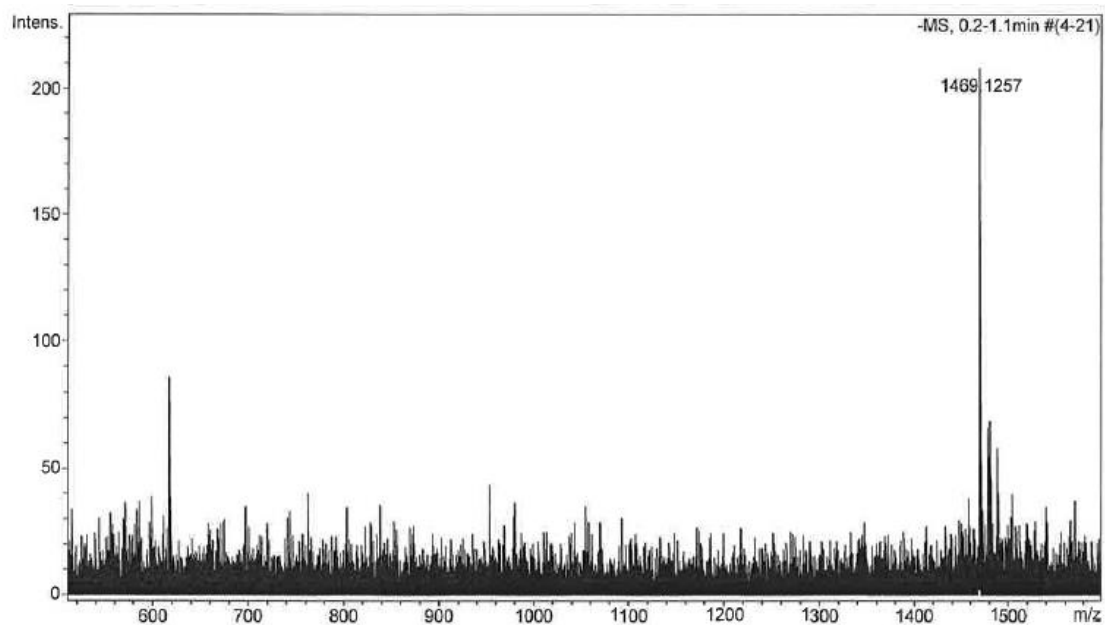
RNA 15 (5'-ACG-3')



S2 ES MS spectra of fragments (2',3'-cyclic phosphates) produced in the cleavage of RNA 3 and 6

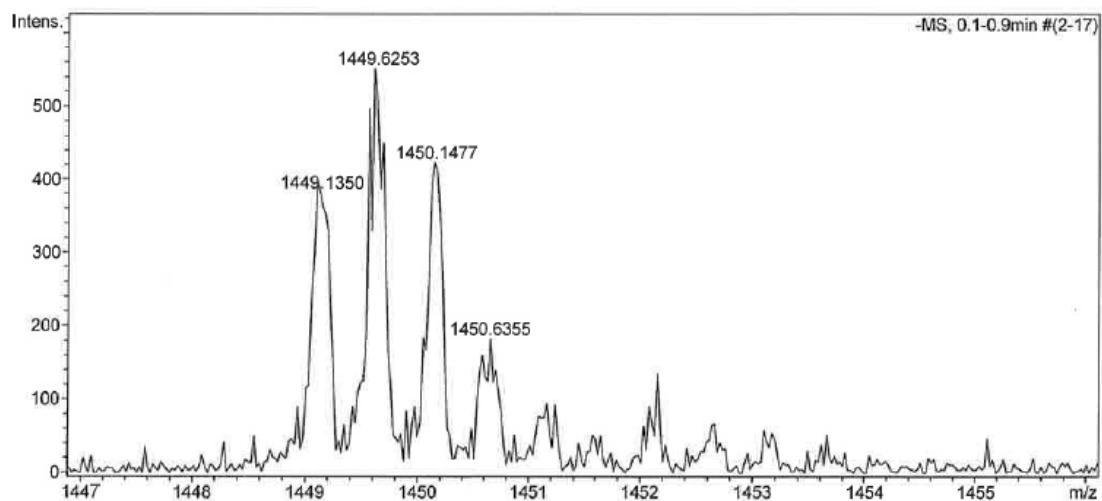
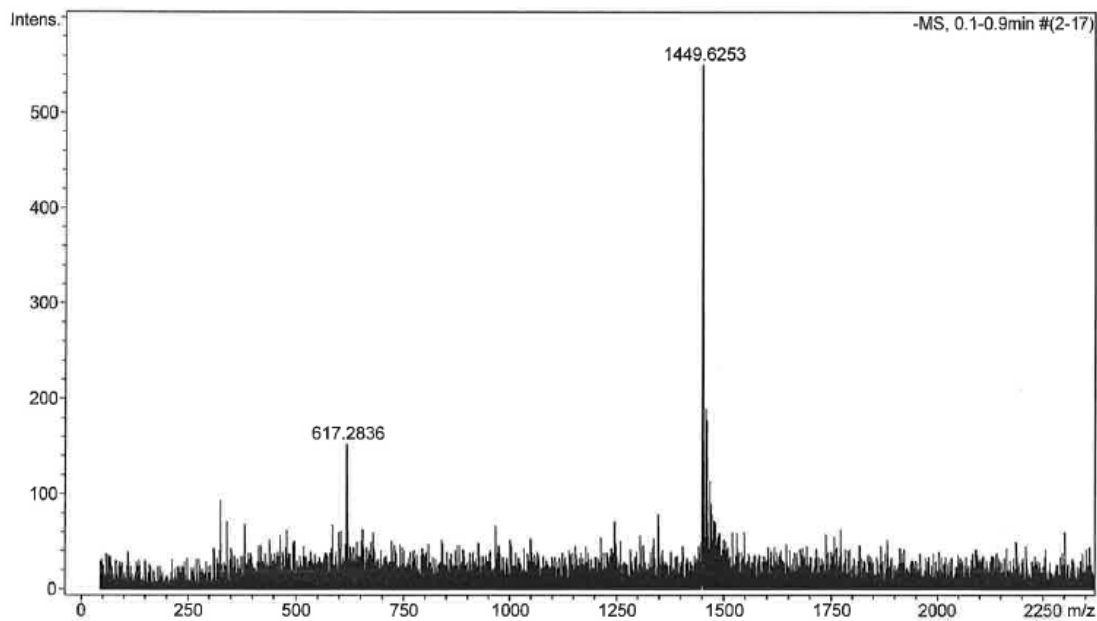
RNA 3 fragment 5'-AGAGUUC-AG-3' cyclic phosphate

$m/z$ :  $C_{87}H_{104}N_{37}O_{62}P_9^{2-}$   $[M]^{-2}$  calcd 1468.7, found 1469.1



RNA 6 fragment 5'-AGAGUUC-UA-3' cyclic phosphate

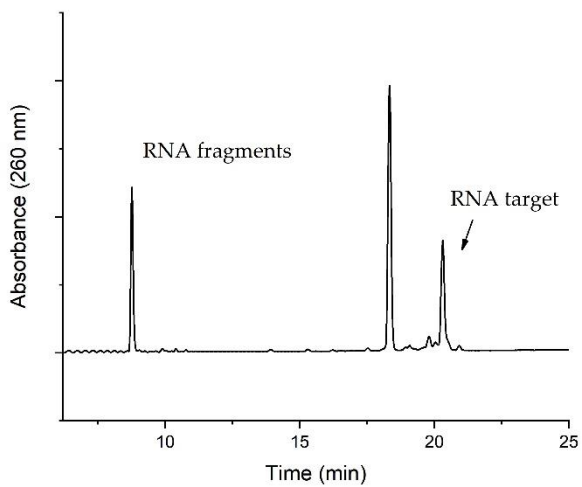
$m/z$ :  $C_{86}H_{103}N_{34}O_{63}P_9^{2-}$   $[M]^{-2}$  calcd 1449.2, found 1449.6



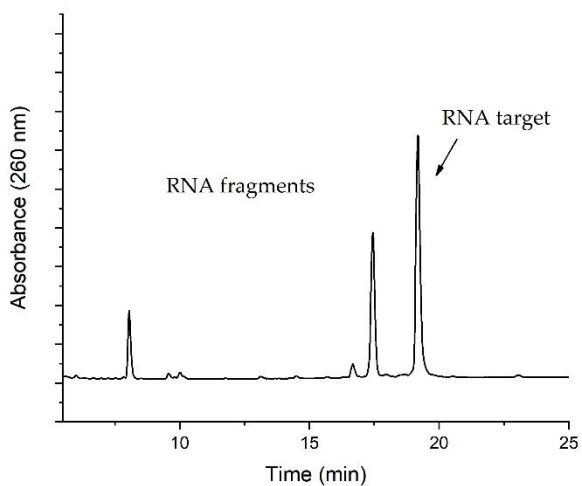


**S3** Cleavage of (or the lack thereof) modified 4-nucleotide bulge-forming RNAs **16** (1 h) and **17-19** (24 h) by PNAzyme **I**, and RNA **16** in the presence of PNA-phenanthrene conjugate (**Y**) (24 h)

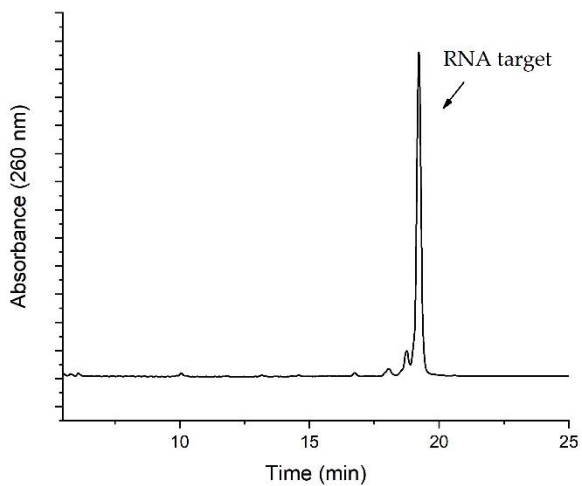
RNA **16** (5'-AUAA-3')



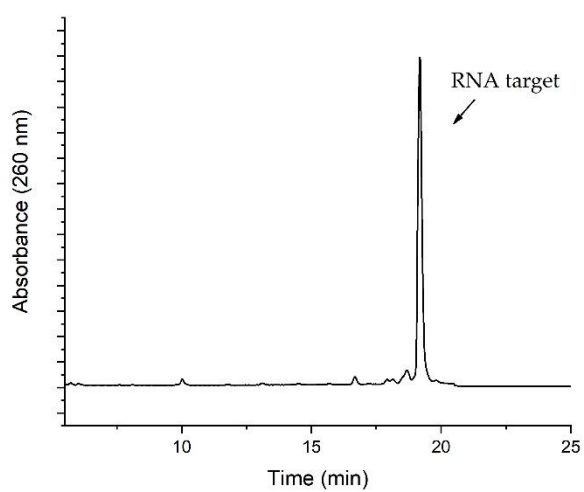
RNA **17** (5'-AUPurA-3')



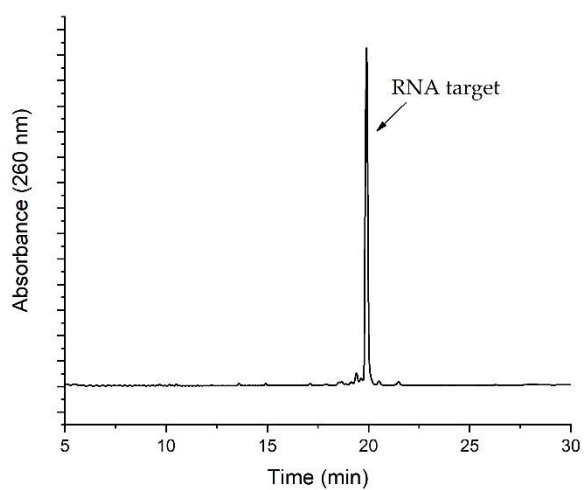
RNA **18** (5'-AUdAA-3')



RNA 19 (5'-AUA<sub>2'</sub>OMeA-3')

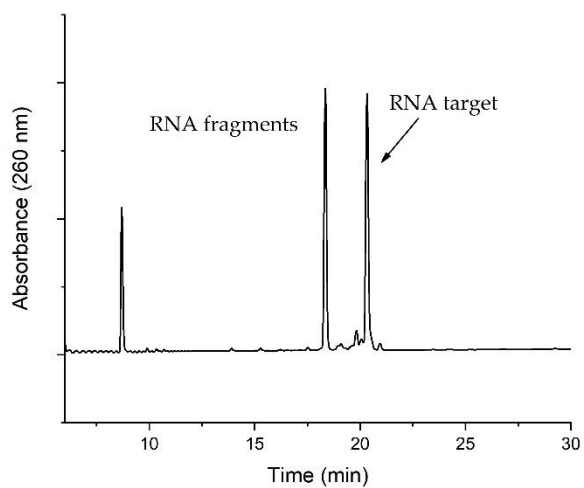


RNA 16 and PNA-phenanthrene conjugate (Y)

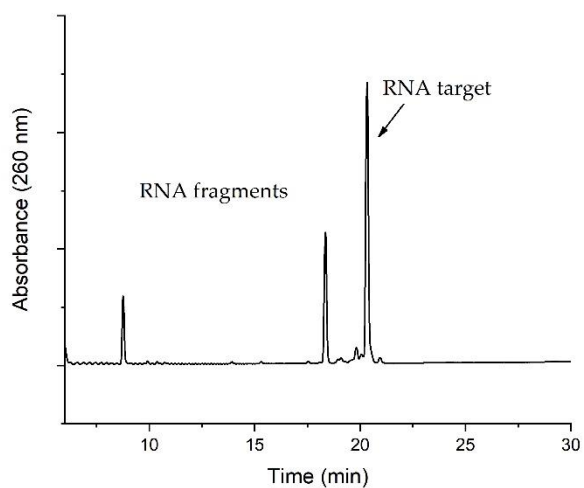


**S4** Cleavage of 4-nucleotides RNAs **16, 20-24** promoted by PNAzyme **I** or **II** (30 min)

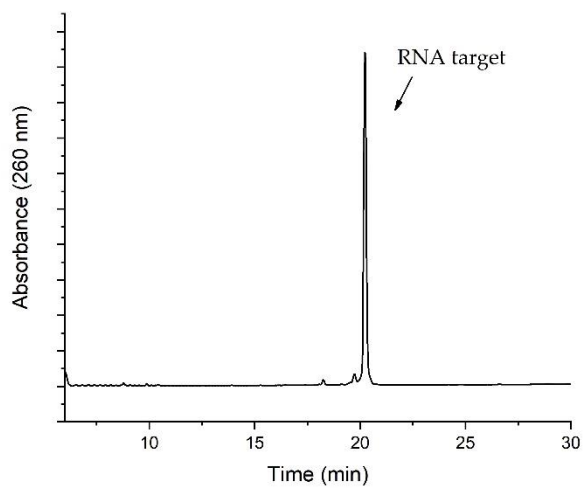
PNAzyme **I**-promoted cleavage of RNA **16** (5'-AUAA-3')



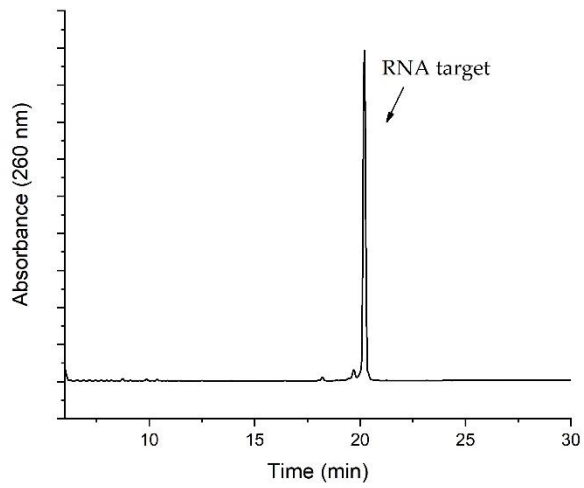
PNAzyme II-promoted cleavage of RNA 16 (5'-AUAA-3')



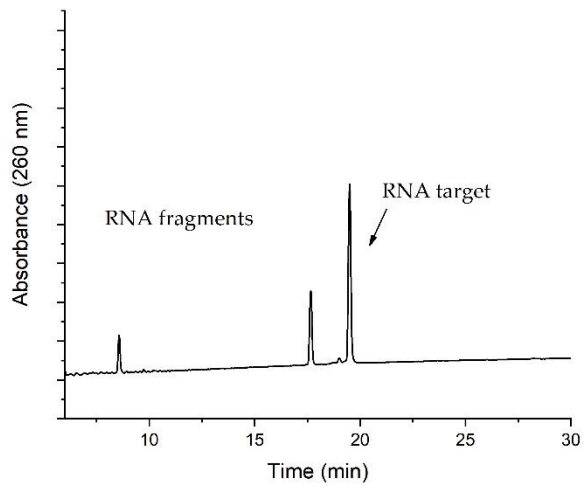
PNAzyme I-promoted cleavage of RNA 20 (5'-AAUA-3')



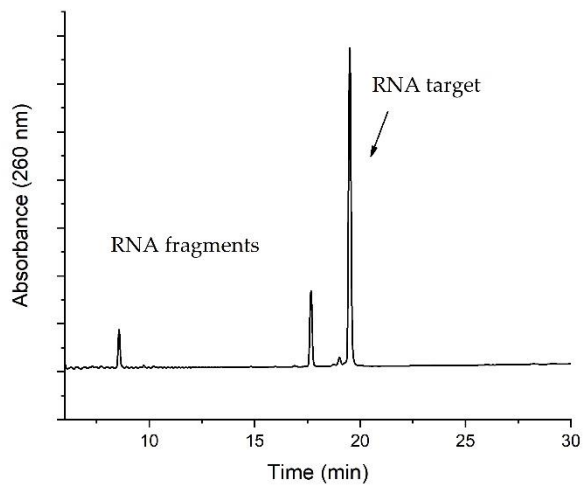
PNAzyme II-promoted cleavage of RNA 20 (5'-AAUA-3')



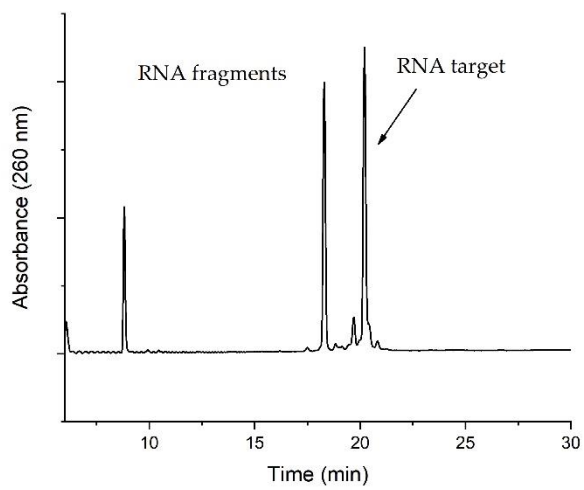
PNAzyme I-promoted cleavage of RNA **21** (5'-AAAA-3')



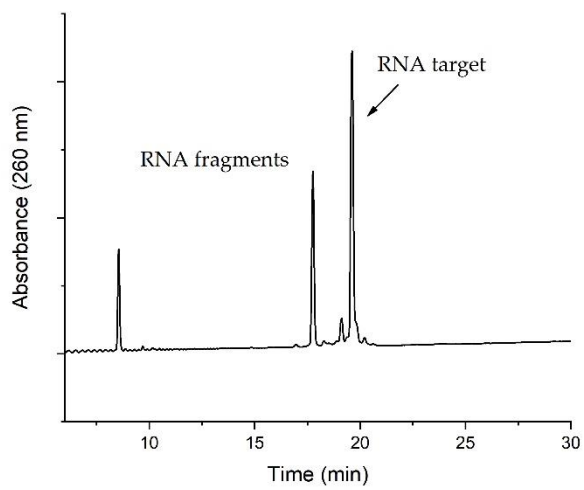
PNAzyme II-promoted cleavage of RNA **21** (5'-AAAA-3')



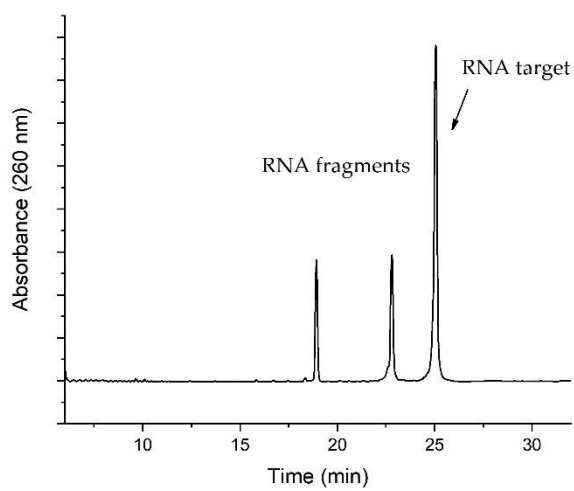
PNAzyme I-promoted cleavage of RNA 22 (5'-ACAA-3')



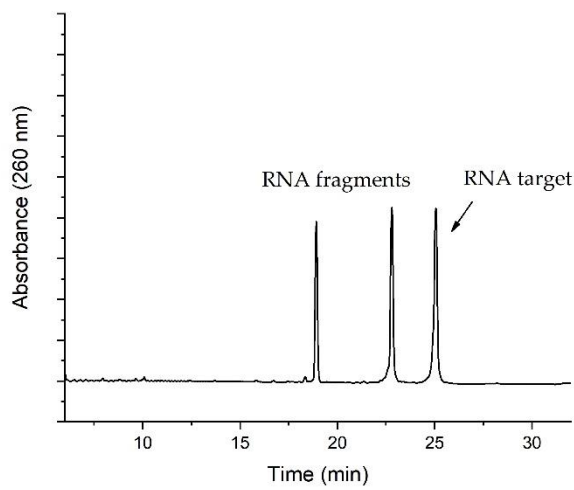
PNAzyme II-promoted cleavage of RNA 22 (5'-ACAA-3')



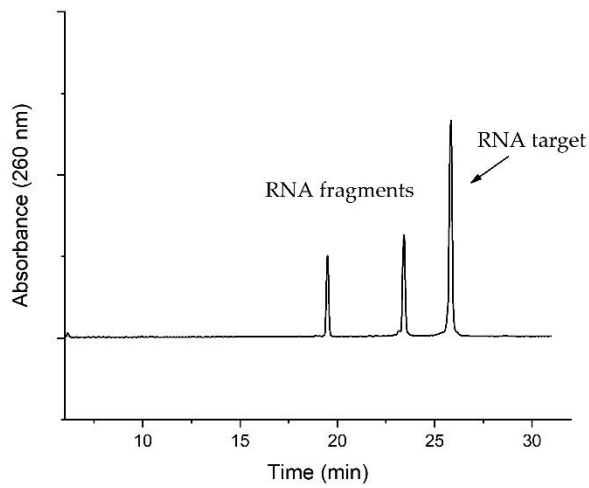
PNAzyme I-promoted cleavage of RNA 23 (36mer 5'-AUAA-3')



PNAzyme II-promoted cleavage of RNA 23 (36mer 5'-AUAA-3')

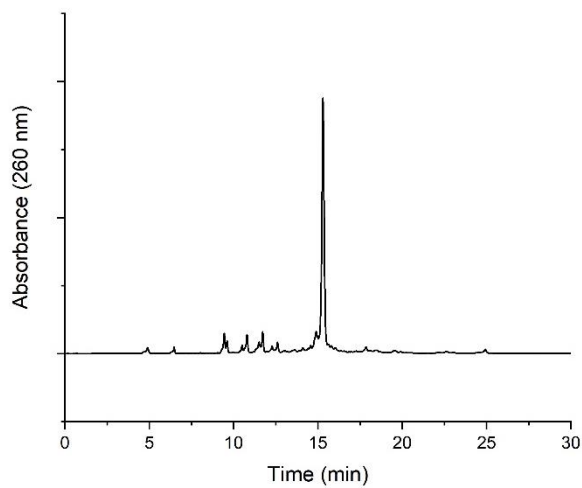


PNAzyme II-promoted cleavage of RNA 24 (36mer 5'-AUAA-3' and 5'-AUAU-3')

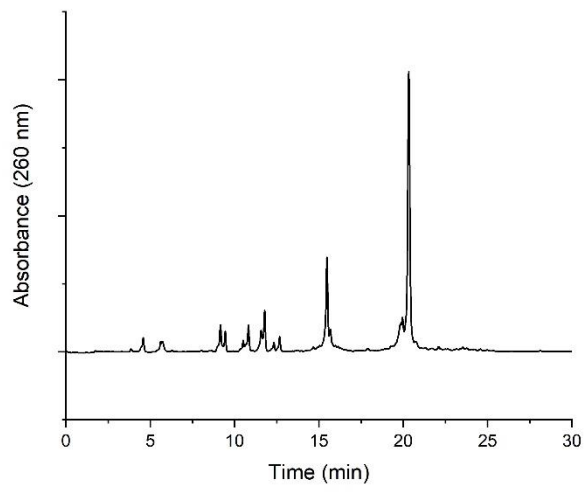


S5 RP HPLC analysis of PNAzyme II

Reference crude unconjugated PNA II:



Crude PNzyme II (post conjugation):



Purified PNzyme II:

