PNA Based Artificial Nucleases Displaying Catalysis with Turnover in Cleavage of a Leukemia Related RNA Model

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Supporting information

bcr/abl RNA model. The bcr/abl RNA model was purchased from Dharmacon and was first purified by semi-preparative IE HPLC equipped with an Dionex NucleoPac PA-100 (9 × 250 mm) column. A linear gradient of 0-40 % buffer B over 30 min was used with a flow rate of 4 ml/min (A) 20 mM NaOAc in 30 % aqueous acetonitrile and (B) 20 mM NaOAc, 0.6 M $LiClO_4$ in 30% aqueous acetonitrile. The collected fractions were lyophilized and then purified with RP HPLC using ODS Hypersil (250 × 10 mm, 5μm) column. A linear gradient of 0-23 % buffer B over 30 min was used with a flow rate of 3 ml/min (A) 50 mM triethylammonium acetate in water (pH 6.5) and (B) 50 mM triethylammonium acetate (pH 6.5) in 50 % aqueous acetonitrile. Purifications of the bcr/abl RNA model were performed at 40 °C. The RNA was lyophilized three times before use and stored as frozen solution. ES-TOF: mass calculated for the bcr/abl RNA model C144H179N61O99P14 [M], 4782; found, 4783.

Thermal melting analysis. Absorbance vs temperature profiles were measured at 260 nm on a Varian Cary 300 UV–Vis dual beam spectrophotometer (Varian). The samples were prepared by mixing two oligonucleotide strands in a 1:1 ratio, 4 μM of each, in a 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA at pH 7.0. Extinction coefficients were calculated from the nearest-neighbour approximation.[1] A termostattable (peltier) multicell (6 + 6) block was used to simultaneously monitor samples. The samples were rapidly heated to 90 ºC, left for 5 min and then allowed to cool to 10 ºC. After equilibration for 10 min at the starting temperature, the dissociation was recorded by heating to 90 ºC at rate of 0.2 ºC/min and data points were collected every 0.1 °C. The Varian Cary WinUV software, version 3 was used to determine the melting temperatures (Tm) by fitting the melting profile to two-state transition model.[1] with linearly sloping lower and upper baselines. Reported values are the average of at least two experiments. Tm(PNAzyme **1**)=55 ºC, Tm(PNAzyme **2**)=55 ºC, Tm(PNAzyme **3**)=52 ºC and Tm(PNAzyme **4**)=54ºC. Observed melting points for PNAzymes **1** and **2** were approximately 10 decrees higher than for the corresponding 2'-*O*-Me systems (Figure S1). Tm(2'-*O*-MeOBAN **1**)=46 ºC and Tm(2'-*O*-MeOBAN **2**)=46 ºC.

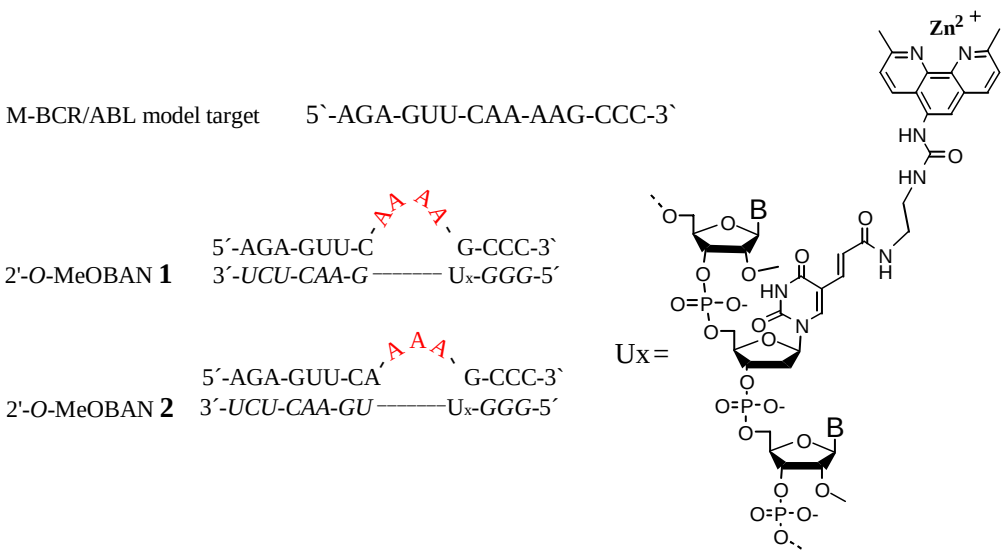


Figure S1. Complexes of 2'-*O*-MeOBAN's **1** and **2** with the M-BCR/ABL RNA model.

HPLC-analysis of turnover reaction with 1:10 ratio of PNAzyme 1 and RNA

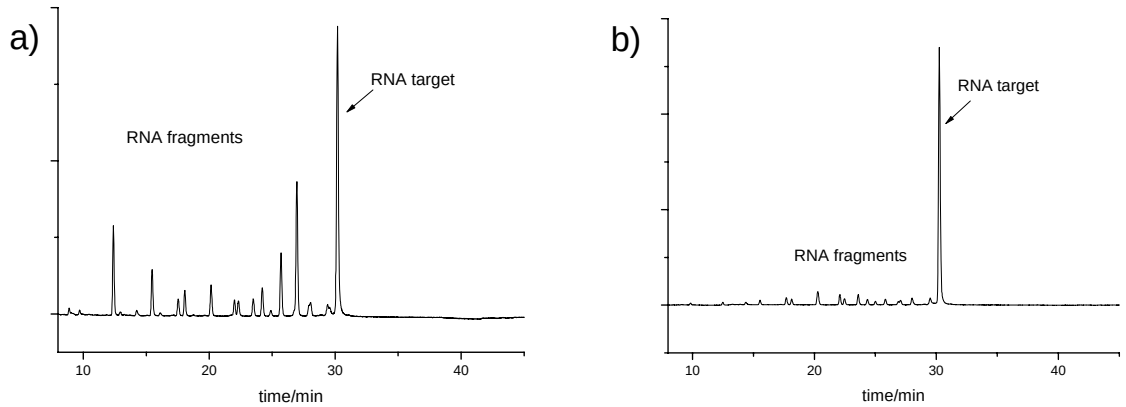
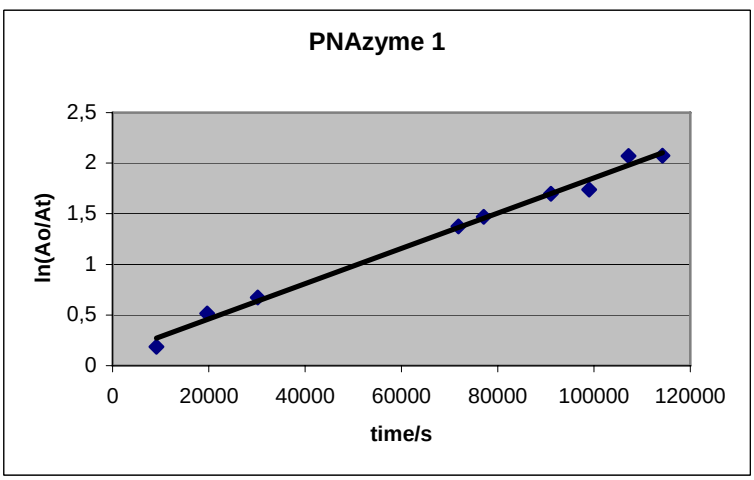
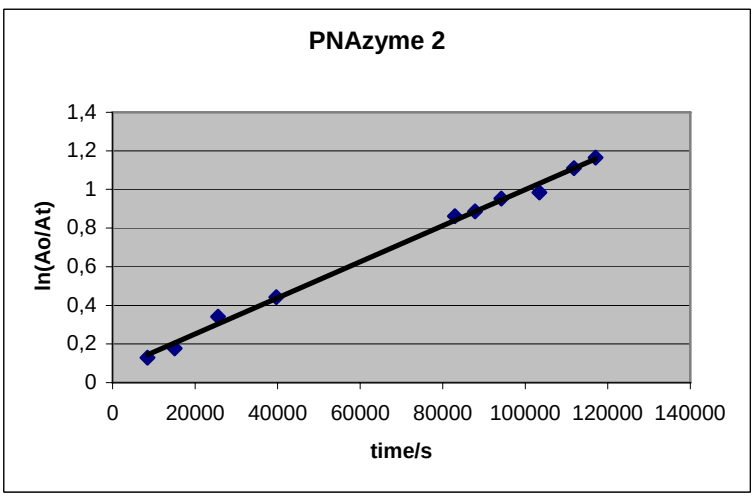
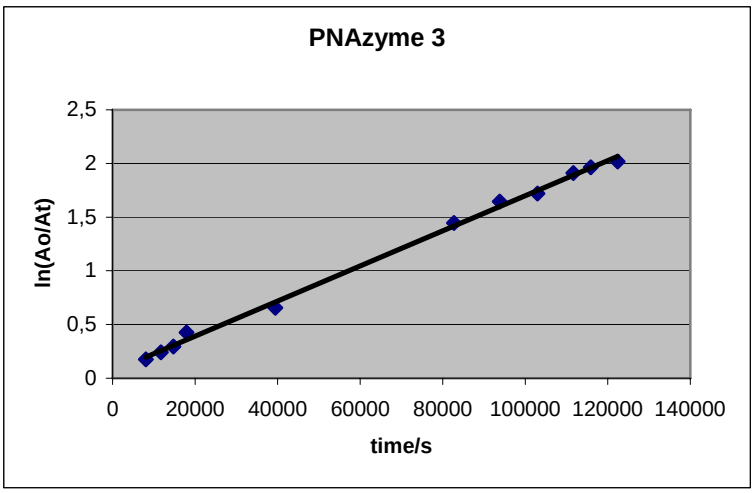


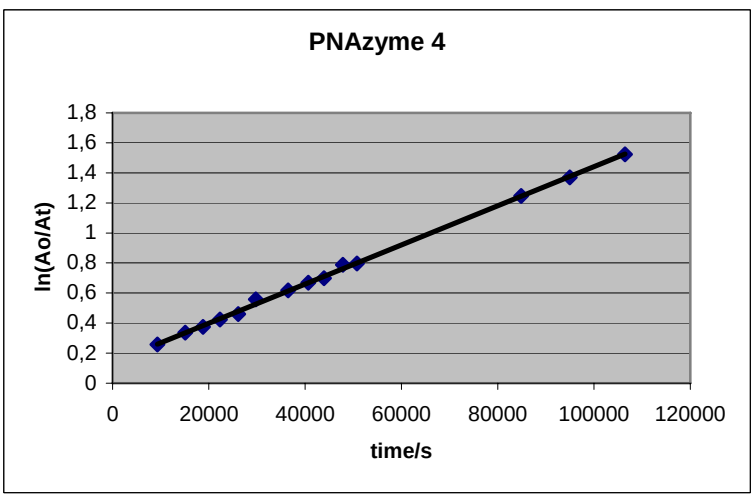
Figure S2. HPLC analysis of reaction in presence of a) PNAzyme **1** and RNA (1:10, PNAzyme:RNA) b) zinc aquo ions and RNA substrate alone. Reactions were carried out using the same effective concentration of Zn^{2+} (50 μM) and were analysed at the same time point (76 h).

Examples of plots from experiments with 1:1 ration of PNA-zyme to RNA for the determination of rate constant
(Rate constants reported in Table 1 were the average of at least 2 such experiments)









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[1] J. D. Puglisi and I. Tinoco, Jr., *Methods Enzymol.* 1989, **180**, 304-325.