Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Simple and sensitive fluorescence detection of the RNA endonuclease activity of mammalian argonaute2 protein based on RNA molecular beacon

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Experimental Section

Materials. Recombinant mammalian Ago2 was obtained from Sino Biological Inc. (Beijing, China). Oligonucleotides were synthesized by SBS Genetech. Co., Ltd. (Shanghai, China). Nuclease inhibitor RNasin, and ribonuclease A (RNase A) were ordered from Promega. P. Diethypyrocarbonate (DEPC) was ordered from amresco (USA). Human hemoglobin, bovine serum albumin, and α-thrombin from human plasma were purchased from Sigma-Aldrich. Other reagents were of analytical reagent grade and used as received. Unless otherwise noted, all solutions were prepared with double-distilled water that was autoclaved. Sequences of guide RNA (5'-UUGUCUCUGGUCCUUAC-3'), RNA molecular beacon (RMB, 5'-FAM-AAGUAAGGACCAGAGACAA-BHQ1-3') and control RMB (5'-FAM-GGUACCUUUGACUCUCAUU-BHQ1-3') were used in this work. The stock solutions of these three oligonucleotides were prepared in 0.1 mM phosphate buffer solution (PBS, pH 7.4) containing 1 ‰ diethyl DEPC (v/v) to remove the interference of RNase in the following experiments.

Instruments. FL measurements were performed using a F-4500 spectrofluorimeter with a scan rate at 1200 nm/min. The excitation wavelength was at 490 nm, and the 24 photomultiplier tube voltage was

700 V. The slits for excitation and emission were set at 5 nm/5 nm.

Fluorescent measurements. Ago2 was first preincubated with guide RNA in Tris buffer solution (20 mM Tris−HCl, 1 unit/μL RNasin, 50 mM KCl, 1.5 mM $MgCl_2$, 1‰ DEPC) at 37 °C for 30 min to allow guide RNA to bind to Ago2. For fluorescent assay of Ago2-induced RNA strand cleavage, 7.5 μL of 2 μM RMB solution, a series of different volumes of the above Ago2 solution were added into the quartz cell for time-dependent FL measurement at λex/λem = 490/520 nm. The final volume of the solution was fixed at 300 μL with the final concentration of RMB of 50 nM. The control experiment was carried out under the same condition without the addition of the above Ago2 solution. All experiments were performed at 37 °C and repeated three times.

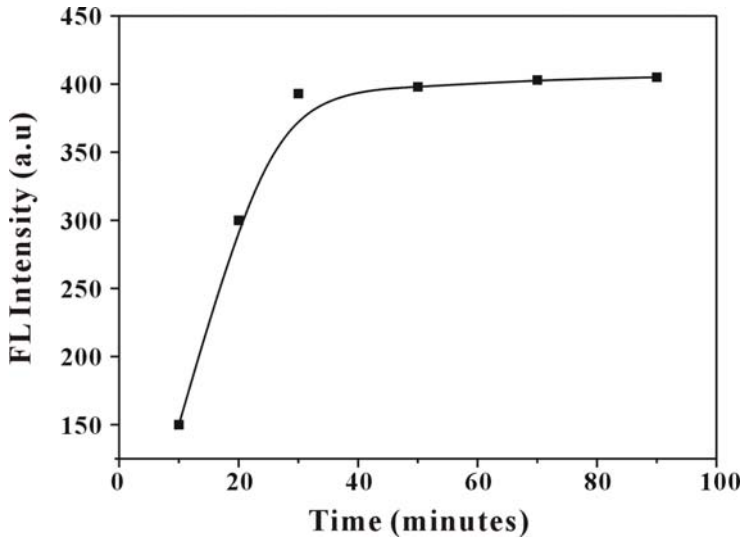

Fig. S1 Plot for the kinetics of the enzymatic reaction following FL intensity changes upon enzymatic reaction in the presence of 25 nM Ago2.

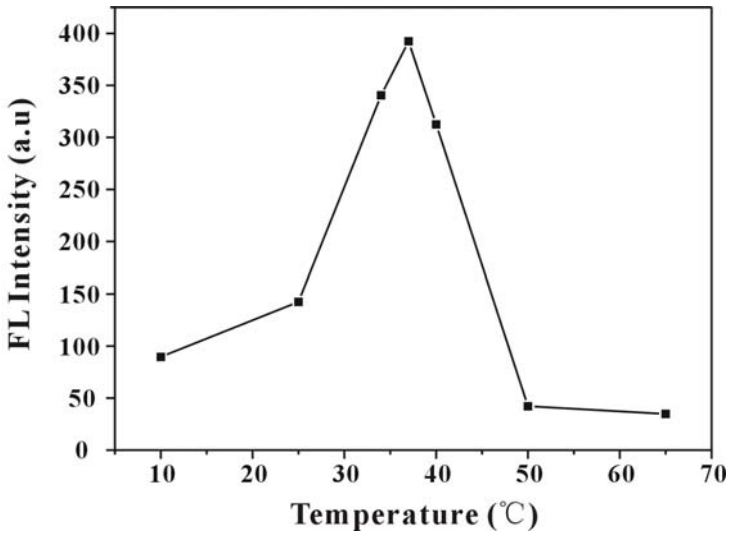

Fig. S2 FL intensity changes of the Ago2 sensing system at different enzymatic reaction temperatures in the presence of 25 nM Ago2.

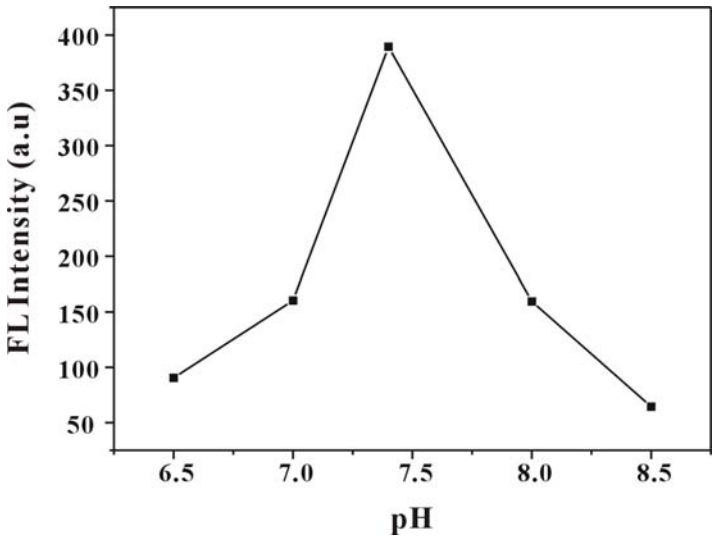

Fig. S3 FL intensity changes of the Ago2 sensing system at enzymatic reaction solutions of different pH in the presence of 25 nM Ago2.

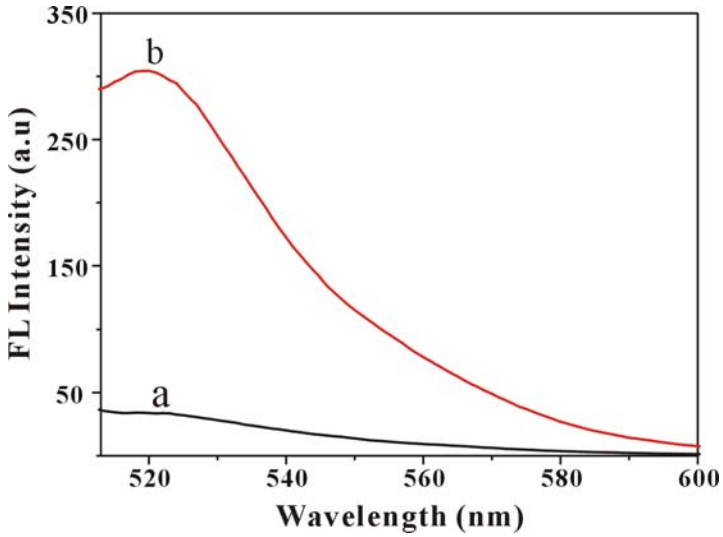

Fig. S4 FL emission spectra of RMB (50 nM) (a) without Rnase A and (b) with 0.5 unit/mL Rnase A, which was conducted in Tris-HCl buffer without 1‰ DEPC and RNasin.