*Supporting Information for*

# Catalytic and Molecular Beacons for Amplified Detection of Metal Ions and Organic Molecules with High Sensitivity

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## 1. Experimental details

**General procedures.** All oligonucleotide samples were purchased HPLC purified from Integrated DNA Technologies, Inc. (Coralville, IA). Lead acetate and all other reagents were of analytical reagent grade, purchased from Sigma-Aldrich Chemical Co., and used without further purification. All solutions were prepared in Milli-Q water (resistance >18 MΩ·cm) from a Millipore system. Fluorescence measurements were performed using a FluoroMax-P spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ) with a ~800 μL glass tube with a ~4 mm pathlength. The excitation wavelength was fixed at 492 nm with both excitation and emission slits set at 2.0 nm. The pH measurements were carried out using an Accumet basic AB15 pH meter (Fisher Scientific, USA).

**Table S1.** Sequences of oligonucleotides used in this work.[a]

| Oligonucleotide | Sequences (from 5′ to 3′) |
|---|---|
| MB1 | /FAM/-CATGCGCGATTGAAATTGACCCACTATrAGGAA-GAGATGTTACGAGGCGCGCGCATG-/BHQ1/ |
| MB2 | /FAM/-CCACCACATTCAAATTCACCAACTATrAGGAAG-AGATGTTACGAGGCGGTGGTGG-/BHQ1/ |
| AD-aptamer-Mgzyme | CATCTCTTCAGCGATCTAGGGGGAGTATTGCGGAGGAT-AGCACCCATGTTAGTTGGT |
| 17DS-FD | /FAM/-ACTCACTATrAGGAAGAGATG-/Dabcyl/ |
| 17E-Dy | CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-/Dabcyl/ |
| 17E-1 | CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGT |
| 17E-2(9+9) | CATCTCTTCTCCGAGCCGGTCGAAATAGTTGGT |
| 17E-2(8+9) | ATCTCTTCTCCGAGCCGGTCGAAATAGTTGGT |
| 17E-2(8+8) | ATCTCTTCTCCGAGCCGGTCGAAATAGTTGG |
| 17E-2(7+8) | TCTCTTCTCCGAGCCGGTCGAAATAGTTGGT |
| 17E-2(7+7) | TCTCTTCTCCGAGCCGGTCGAAATAGTTGG |

[a] The underlined sequences in MB sequence represent the stem of MB, rA denotes adenosine ribonucleotide at that position while all others are deoxyribonucleotides.

The original 8-17 DNAzyme catalytic beacon system consists of 17DS-FD and 17E-Dy with their sequences shown in Table S1. The sequences of other oligonucleotides used in this work are also given

in table S1. In table S1, the DNA 17E-1 denotes the DNAzyme for MB1; 17E-2(9+9), 17E-2(8+9), 17E-2(8+8), 17E-2(7+8), 17E-2(7+7) corresponds to the DNAzyme for MB2 with different lengths of the arm sequences.

**Kinetic fluorescence studies.** To detect $Pb^{2+}$ using the CAMB, 200 nM of the 8-17 DNAzyme strand with 9 bases on each arm (17E-2(9+9)) (Table S1 for sequence information) was first incubated with 300 nM MB2 at room temperature for 20 min in a buffer solution containing 25 mM HEPES (pH 7.0) and 100 mM NaCl. A 95 μL aliquot of the above hybridized DNA sample was transferred into a glass tube, which was then placed in the fluorometer thermostated at 25°C. The detection was carried out in kinetics mode. The excitation and emission were set at 492 nm and 518 nm respectively, and the fluorescence change was monitored for 600 s with time intervals of 15 s. After the initial reading, the glass tube was taken out, and a 5 μL aliquot of concentrated metal ions stock solution was added to induce the DNAzyme catalyzed cleavage reaction. After vortexing to mix the components thoroughly, the tube was quickly put back into the fluorometer to continue the kinetics measurement.

The kinetic assays for amplifying detection of adenosine were performed with 95 μL of a solution containing 300 nM MB2, 200 nM AD-aptamer-Mgzyme, 10 mM $Mg^{2+}$, 25 mM HEPES (pH 7.2) and 100 mM NaCl. The mixed solution was annealed by heating to 90 °C for 5 min and subsequently cooling to 4 °C slowly. The annealed sample was then incubated at room temperature for 20 min. The mixed solution in the glass tube was then monitored on the fluorometer for 1200 s after addition of adenosine with a time interval set at 30 s.

**Fluorescence assay procedures for optimization purpose.** To probe the effect of the MBs structure on the sensing performance of the catalytic and molecular beacon (CAMB) system, 17E-2(9+9) at 200 nM was first incubated with different MBs (MB1 and MB2, separately) at 200 nM at room temperature for 20 min in the buffer containing 25 mM HEPES (pH 7.0) and 50 mM NaCl. A 95 μL aliquot of the hybridized DNA sample was transferred in a quartz cuvette, which was then placed in a fluorometer thermostated at 25°C. The kinetics of the fluorescence increase was recorded. The excitation and

emission wavelengths were set at 492 nm and 518 nm respectively, and time interval was chosen at 15 s for a total measuring time of 600 s. After the initial reading, the quartz cuvette was taken out, and a 5 μL aliquot of 10 μM $Pb^{2+}$ was added to induce the cleavage reaction. After vortexing, the cuvette was immediately put back into the fluorometer to continue the kinetics assay. Control experiments for the fluorescence change of MB1 and MB2 in the absence of DNAzyme were also carried out following the procedure shown above. To investigate the effect of the arm length of the DNAzymes on the sensing performance of the system, MB2 with at 200 nM was first incubated with 200 nM DNAzymes with different arm lengths (17E-2(9+9), 17E-2(8+9), 17E-2(8+8), 17E-2(7+8), 17E-2(7+7)) individually, at room temperature for 20 min in the buffer containing 25 mM HEPES (pH 7.0) and 50 mM NaCl. Fluorimeter was then used to record the fluorescence change by following the procedure shown above. Similar procedure was also used to investigate the effect of pH values with buffer solutions of different pHs with 100 mM NaCl, as well as the effect of salt concentrations with buffer solution (pH 7.0) containing different concentrations of NaCl.

**Gel electrophoresis.** A mixture solution (45 μL) containing 1μM 17E-2(9+9), varing concentrations of MB2 (0, 1, 3, or 6 μM), 25 mM HEPES (pH 7.0) and 100 mM NaCl was incubated at room temperature for 20 min. A 20 μM $Pb^{2+}$ was then added into the mixed solution to initiate the cleavage reaction. After 30 min 's reaction, 10 μL of the mixed solution were then transferred into 10 μL of stop buffer (200 mM EDTA and 8 M urea) to quench the cleavage reaction. The uncleaved and cleaved substrates were separated by denaturing polyacrylamide gel electrophoresis, and then analyzed by a fluorescence image scanner (FLA-3000G; Fuji, Tokyo, Japan) using 473 nm excitation.

## 2. Supplementary figures

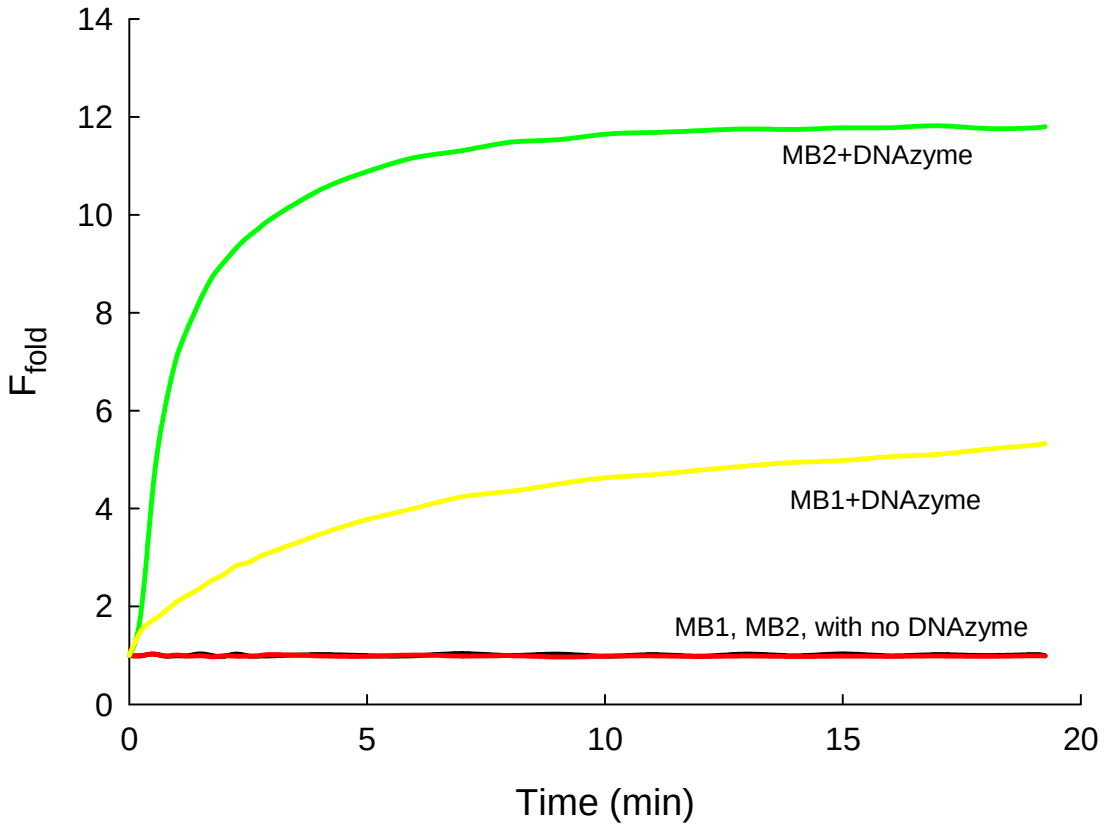


**Figure S1.** Time-dependent fluorescence enhancement of the CAMBs with different MBs as substrates in the presence of 10 μM $Pb^{2+}$. The concentration of both DNAzyme and substrate is 200 nM, and the buffer contained 25 mM HEPES (pH 7.0) and 50 mM NaCl.

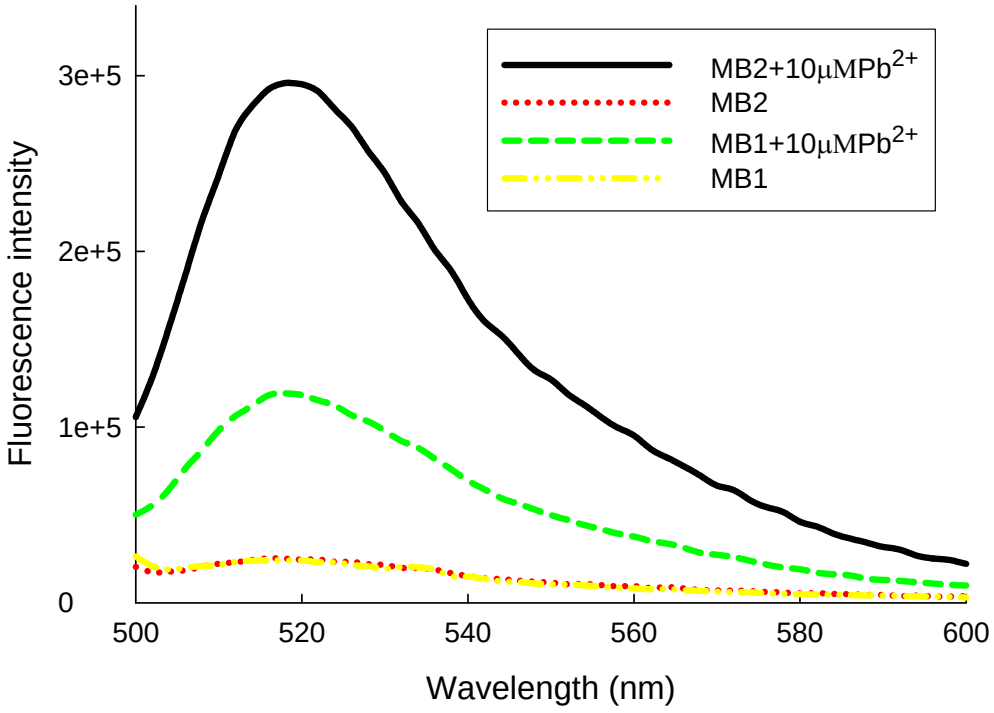


**Figure S2.** Fluorescence spectra of the CAMB system using different MB substrates (MB1 or MB2) before, and after 10 μM $Pb^{2+}$ induced cleavage reaction for 30 min. The concentration of both DNAzyme and substrate is fixed at 200 nM, and the buffer contained 25 mM HEPES (pH 7.0) and 100 mM NaCl.

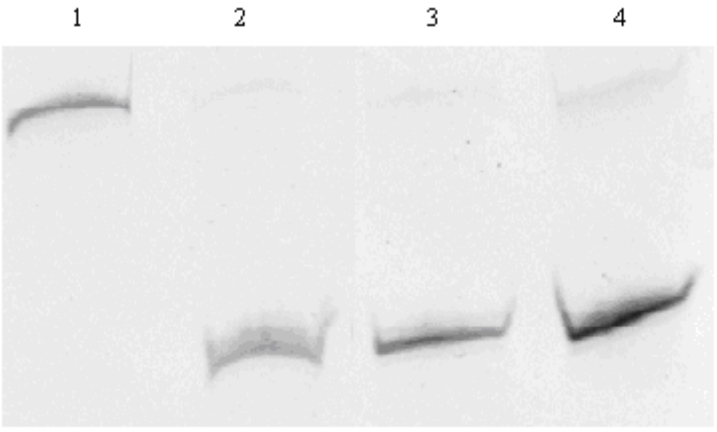


**Figure S3.** Multiple-turnover catalytic activity of CAMB system by using the 8-17 DNAzyme and MB substate (MB2). Gel electrophoresis image assay of the 8-17 DNAzyme. Lane 1, MB2 alone with 20 μM $Pb^{2+}$; lanes 2-4: 30 min after addition of 1, 3 and 6 μM of MB2 into 1 μM 8-17 DNAzyme in the presence of 20 μM $Pb^{2+}$.

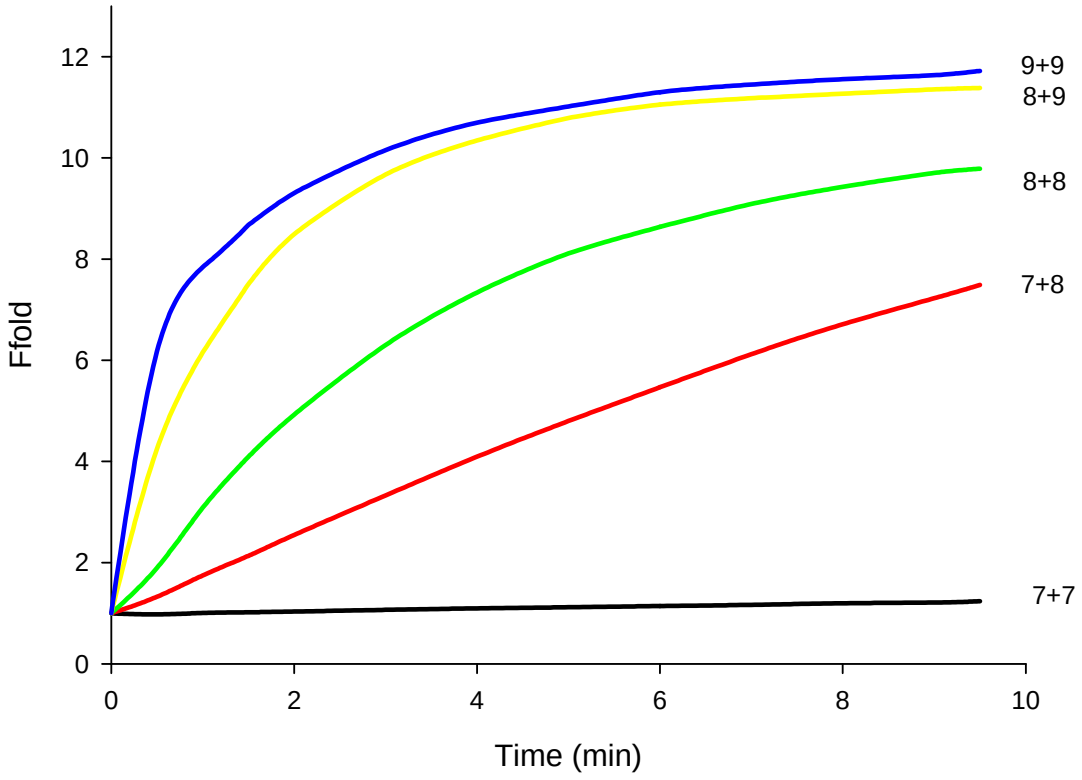


**Figure S4.** Time-dependent fluorescence enhancement of CAMB system with DNAzyme strands of different arm lengths and MB2 as a substrate in the presence of 10 μM $Pb^{2+}$. The concentration of both DNAzyme and substrate is fixed at 200 nM, and the buffer contained 25 mM HEPES (pH 7.0) and 50 mM NaCl.

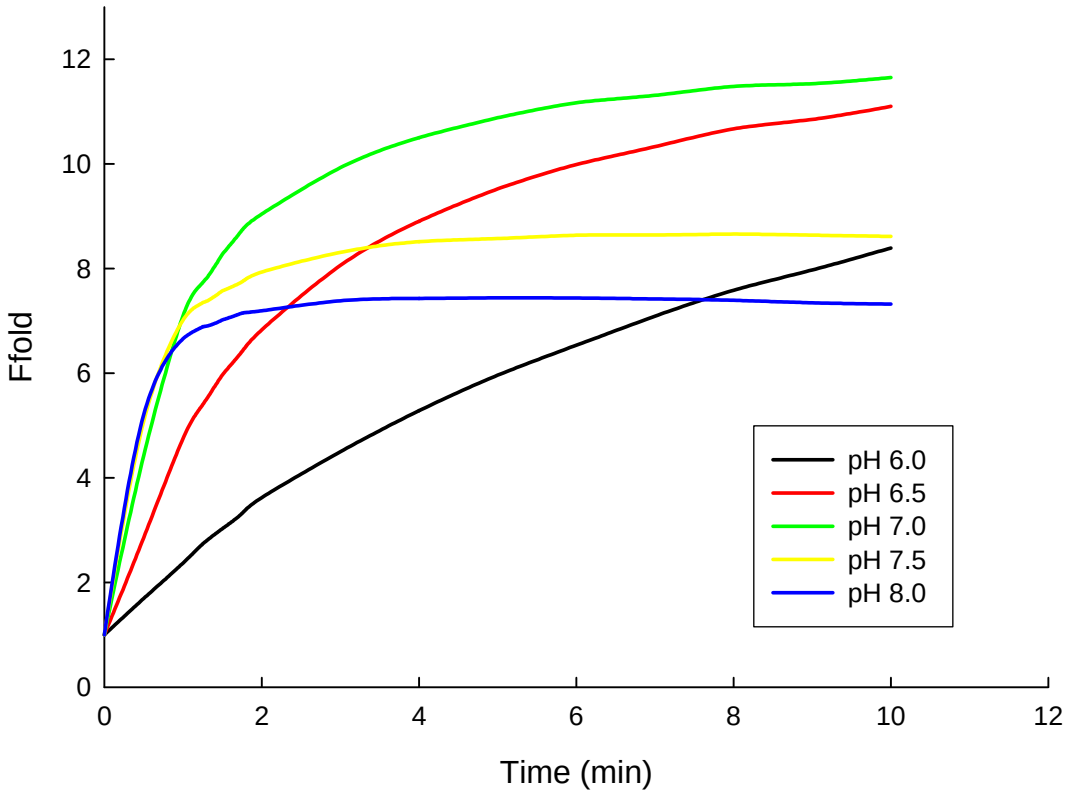


**Figure S5.** Time-dependent fluorescence enhancement of CAMB system under different pHs in the presence of 2.0 μM $Pb^{2+}$. The concentration of both DNAzyme and substrate is fixed at 200 nM, and the buffer contained 25 mM HEPES, and 100 mM NaCl.

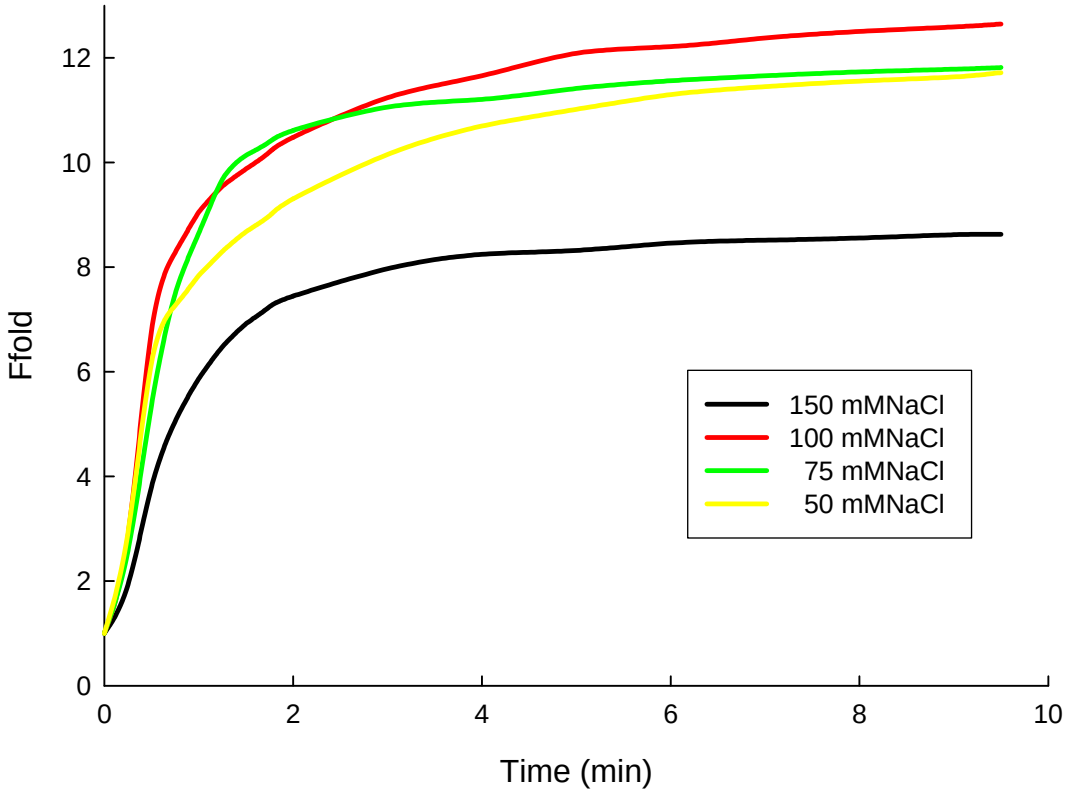


**Figure S6**. Time-dependent fluorescence enhancement of CAMB system with different concentrations of NaCl in the presence of 10 μM $Pb^{2+}$. The concentration of both DNAzyme and substrate is fixed at 200 nM, and the buffer contained 25 mM HEPES (pH 7.0).

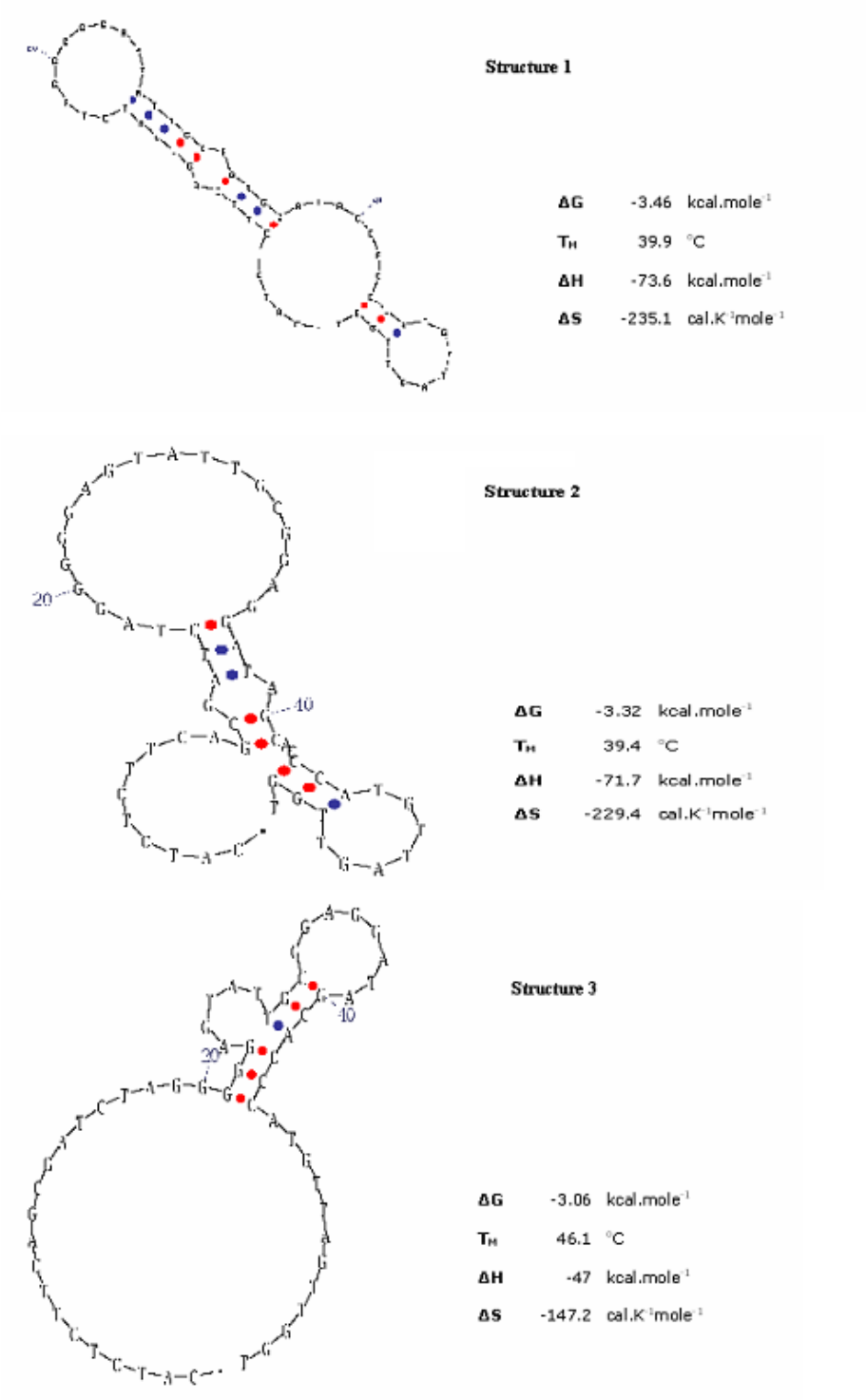


**Figure S7.** Three main stable secondary structures for AD-aptamer-Mgzyme predicted by the Mfold program