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# Supporting Information

# A Novel Nucleic Acid Aptamer Tag: A Rapid Fluorescent Strategy Using A Self-constructing G-quadruplex From AGG Trinucleotide Repeats

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**Materials methods and instrumentation**

The unmodified oligonucleotides were synthesized and purified with PAGE by Sangon Biochemistry Co., Ltd. (Shanghai, China). PYG3, the probe strand containing a PyU at the 5`-end, was a gift from Prof. Byeang Hyean Kim (Pohang University of Science and Technology). Human breast cancer (MCF-7) cells, Chinese hamster ovary (CHO) cells and HeLa cells were all from China Center for Type Culture Collection. The Dulbecco's modified Eagle's medium (DMEM; Hyclone, China) and the fetal bovine serum was bought from Sijiqing Biological Engineering Materials Corporation (Hangzhou, China). The formalin-fixed paraffin-embedded human breast cancer tumor tissue and human benign breast tissue specimens were provided by Zhongnan Hospital of Wuhan University. The water used for all stock and buffer solutions had been purified with the RU Water Purification System (Millipore, Billerica, MA, USA). All the Fluorescent Emission Spectra were collected on a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The experiment about circular dichroism were all carried out on a Chirascan™ CD spectroscopy (Applied Photophysics, Leatherhead, United Kingdom). The Confocal fluorescence microscopy imaging were carried on PerkinElmer UltraVIEW VoX system (PerkinElmer, Boston, United States of America)

**The construction of G-quadruplex**

Unless otherwise specified, the G-quadruplexes of the AS1411 and the (3+1) intermolecular G-quadruplexes formed by the AGG-tri-nucleotide tag and PYG3 were all constructed in $Na^+/K^+$ buffer (NaCl 100mM, KCl 10mM).

**Fluorescence measurements**

All spectra were collected on the Hitachi F-4500 fluorescence spectrophotometer by scanning the emission fluorescence signal from 400 to 750 nm with an excitation wavelength of 385 nm. The excitation and emission slit widths were 10/10 nm. For the kinetic fluorescence measurements, the excitation wavelength was kept at 385 nm, and the emission fluorescence signal was measured at 450 nm.

**Circular dichroism measurements**

The circular dichroism spectra were collected from the Chirascan$^{TM}$ CD spectrometer by scanning from 220 to 345 nm at a speed of 200 nm/min. The response time was 2 s, and the bandwidth was 5 nm. All CD spectra were baseline-corrected for signal contributions due to the buffer. Unless otherwise specified, all circular dichroism measurements were carried out at ambient temperature.

**Thermodynamic melting research**

The structural changes of nucleic acids as the temperature increases could be monitored by the Chirascan$^{TM}$ CD spectroscopy equipped with a temperature controller. The temperature was increased from 4 to 90 °C at a speed of 0.5–1.5 °C/min. The bandwidth was 1 nm. Melting curves were obtained by monitoring the molar ellipticity at 260 nm, which was the characteristic band for G-quadruplexes in this paper. Melting profiles were analysed by fitting them to a concerted two-state model. The sigmoidal fit was performed for the melting curves in Origin 8.0 software. The melting temperatures were calculated by a logistic equation.

**Cell culture**

MCF-7 cells, CHO cells and HeLa cells were all maintained in Dulbecco's modified Eagle's medium with 10% foetal bovine serum and 1% penicillin-streptomycin. All cells were incubated in a humidified 37 °C incubator supplied with 5.0% $CO_2$.

**Confocal fluorescence microscopy imaging in living cells**

For confocal fluorescence microscopy imaging, MCF-7 and CHO cells were seeded in a 35 mm confocal dish (Nest, China) for 24 h. When the cells reached approximately 30% confluence, they were washed three times with a 10% PBS buffer. Then, TG-AS1411 was introduced into the cellular environment (150 nM). After incubation for 40 min at 37 °C, PYG3 was introduced into the cellular environment (150 nM). The cells used with the bioimaging system were maintained at 37 °C for 30 min. The cells were then washed with PBS buffer three times and prepared for confocal imaging.

**Confocal fluorescence microscopy imaging of FFPE tissue sections**

Formalin-fixed, paraffin-embedded specimens were from human breast cancer tumour tissue and human benign breast tissue. First, 3–4 μm thick serial sections were cut from every specimen. After the xylene dewaxing process, the samples were washed in a conventional ethanol gradient and then immersed in water to be rehydrated. The samples were then incubated in 3% $H_2O_2$ for 10 min, washed twice with 10% PBS buffer and maintained in phosphate-EDTA buffer (pH 9) for antigen retrieval. The system was safely heated to 120 °C for antigen retrieval for 5 min and cooled down to room temperature. Then, the prepared samples were immersed in the solution containing TG-AS1411/PYG3 (150 nM) at 37 °C for 40 min. After incubation, the samples were washed with PBS buffer and water and then sealed with neutral resin.

**Cytotoxicity assay**

The cytotoxicity of our strategy was evaluated by MTT assay. HeLa cells were seeded in a 96-well plate. There were approximately 5000 HeLa cells in each test well with fresh cell culture medium. After a 24 hour incubation, the primary cell culture medium was removed and replaced with medium containing different concentrations of TG-AS1411/PYG3 (0 nM, 100 nM, 250 nM, 500 nM, 750 nM, 1 μM). After a 6-hour incubation, 10 μM MTT solution (5 mg/mL in PBS buffer) was added to each well. The 96-well plate was maintained at 37 °C for 4 hours. Then, a 10% SDS solution in 10 mM HCl was added to each well. After a 6-hour incubation, the 96-well plate was subject to absorption measurements at 570 nm by the microplate reader.

**Hairpin structure prediction**

The switching hairpin structures of nucleic acid strands, which were used to develop the AMP biosensor, were predicted by the RNAfold20 program[43] (at http://rna.tbi.univie.ac.at/). The correct folding and energy parameters were evaluated.

**Table S1.** Sequences of oligomers used in the study.

| Oligomer | Sequence(from 5'to 3') |
|---|---|
| TG-AS1411 | GGTGGTGGTGGTTGTGGTGGTGGTGGTTTCCCTTTCAGGAGGAGGA |
| AS1411 | GGTGGTGGTGGTTGTGGTGGTGGTGG |
| AMP-TG-hairpin_1 | AGGAGGAGGAAACCTTCCTGGGGGAGTATTGCGGAGGAAGGTTTCCT |
| AMP-TG-hairpin_2 | CCTAACCTTCCTGGGGGAGTATTGCGGAGGAAGGTTAGGAGGAGG |
| AMP-TG-hairpin_3 | CCTCCTGGGGGAGTATTGCGGAGGAAGGTTCCCAGGAGGAGG |
| AMP-TG-hairpin_4 | CCTGGGGGAGTATTGCGGAGGAAGGTTCCCCAGGAGGAGG |
| AT12 | ATATATATATATATATATATATAT |
| GC12 | GCGCGCGCGCGCGCGCGCGCGC |
| AT24 | ATATATATATATATATATATATATATATATATATATATATATATATAT |
| GC24 | GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC |
| L-5LTR | GGCTCAGGCTAGG |
| i-motif | CCCTAACCCTAACCCTAACCC |

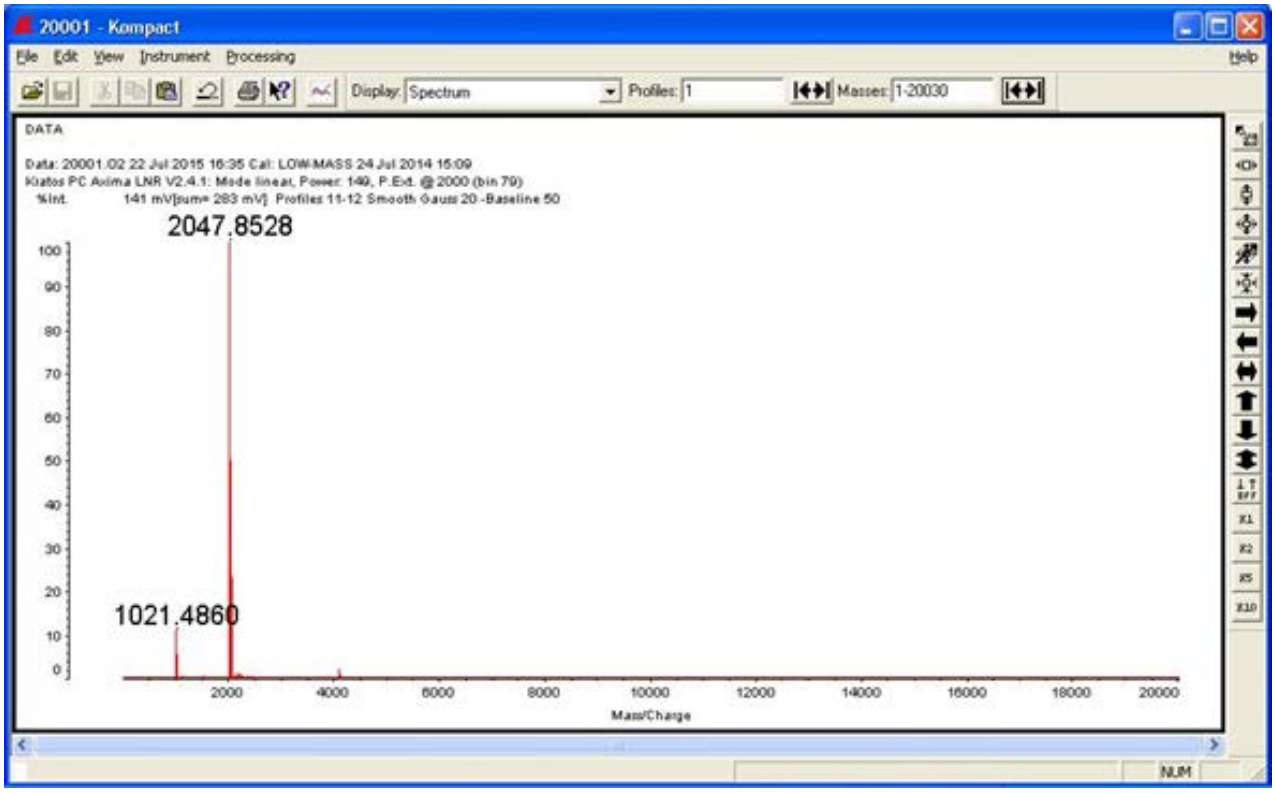


**Figure S1.** MALDI-TOF mass spectral of PYG3

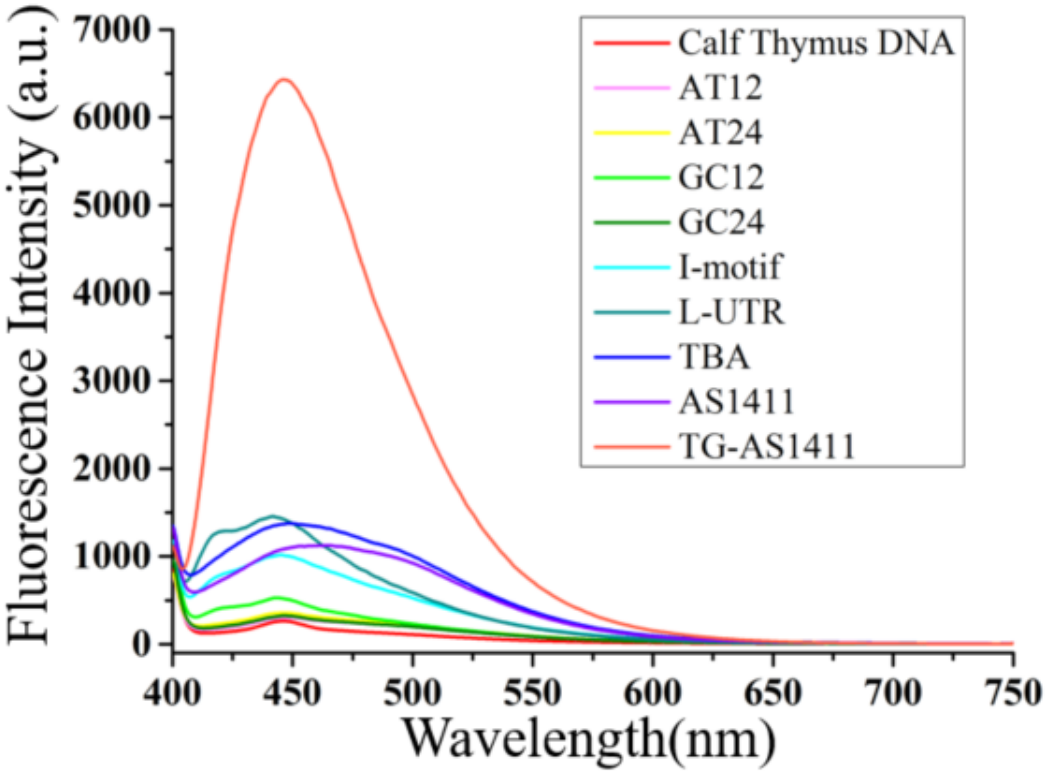


**Figure S2.** The fluorescence emission spectra of PYG3 (1 μM) after incubation with various oligonucleotides (1 μM) in $Na^+/K^+$ buffer at room temperature

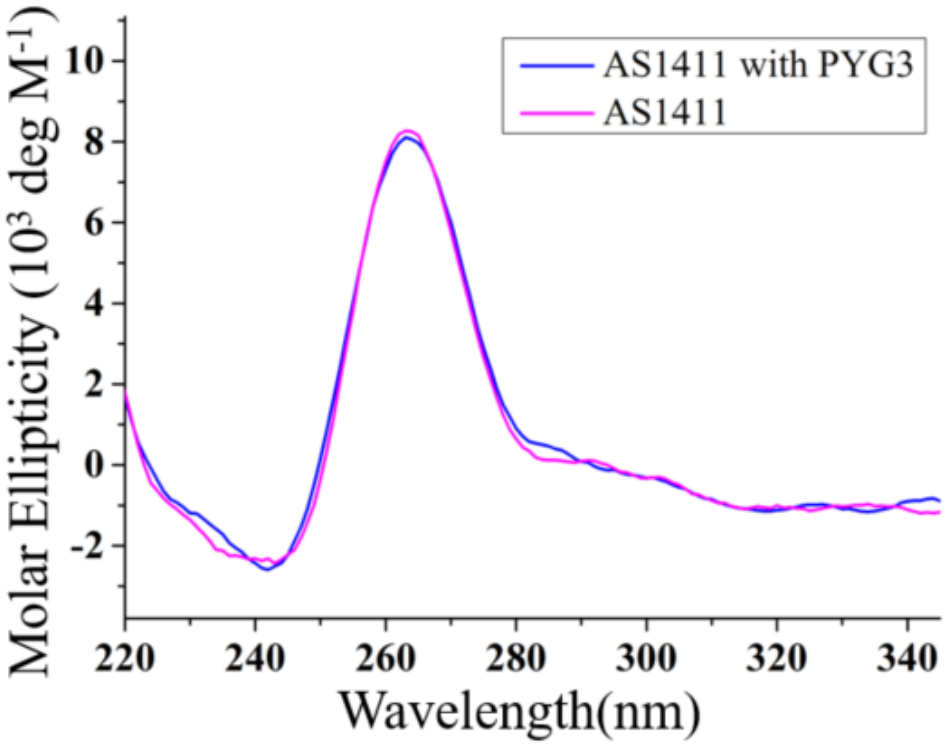


**Figure S3.** The circular dichroism spectra of AS1411 (10 μM) with/without PYG3 (10 μM) in $Na^+/K^+$ buffer

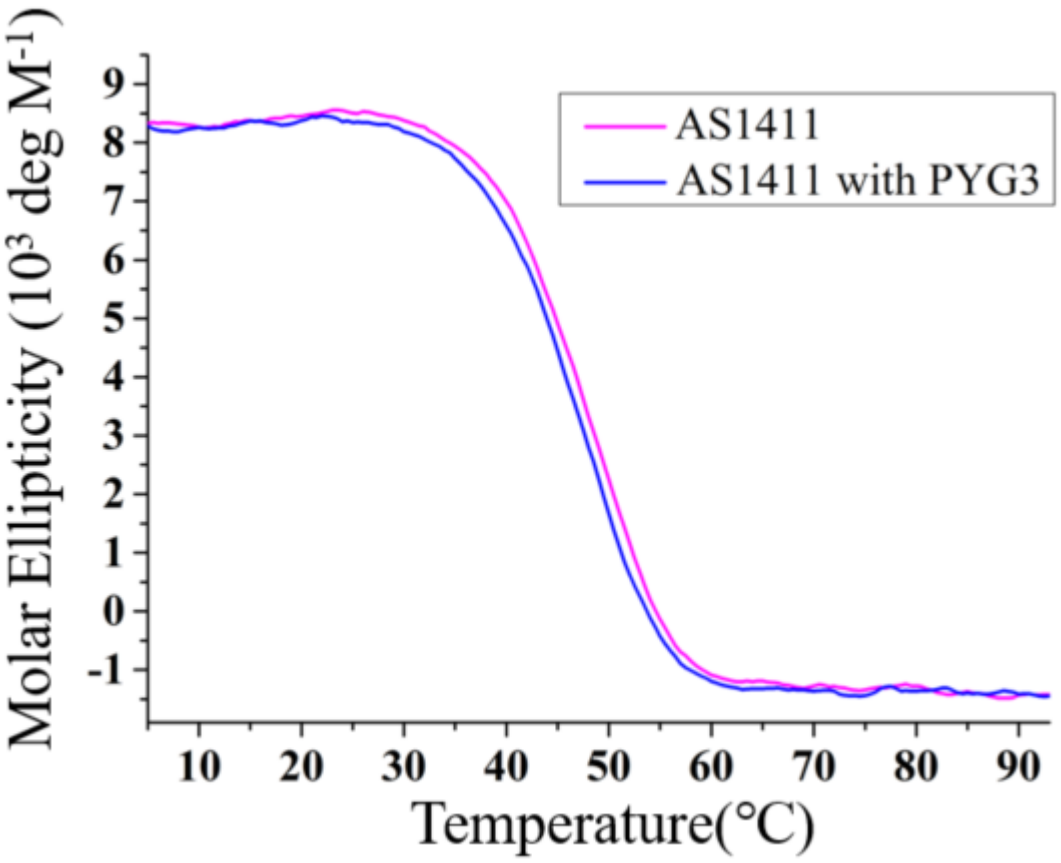


**Figure S4.** The melting curves of AS1411 (10 μM) with/without PYG3 (10 μM) in $Na^+/K^+$ buffer

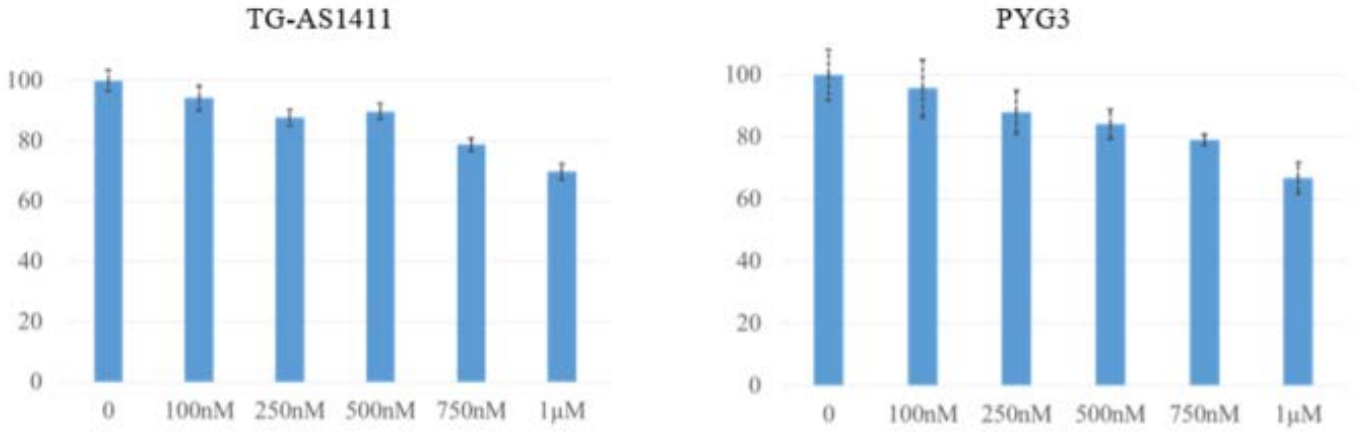


**Figure S5.** The cytotoxicity of the nucleic acid aptamers with AGG-tri-nucleotide tag and PYG3 were evaluated by MTT assay. The HeLa cells were treated with TG-AS1411, and PYG3 at five different concentrations (100nM, 250nM, 500nM, 750nM and 1μM), respectively. With blank control groups, all samples were incubated for 24 hours.

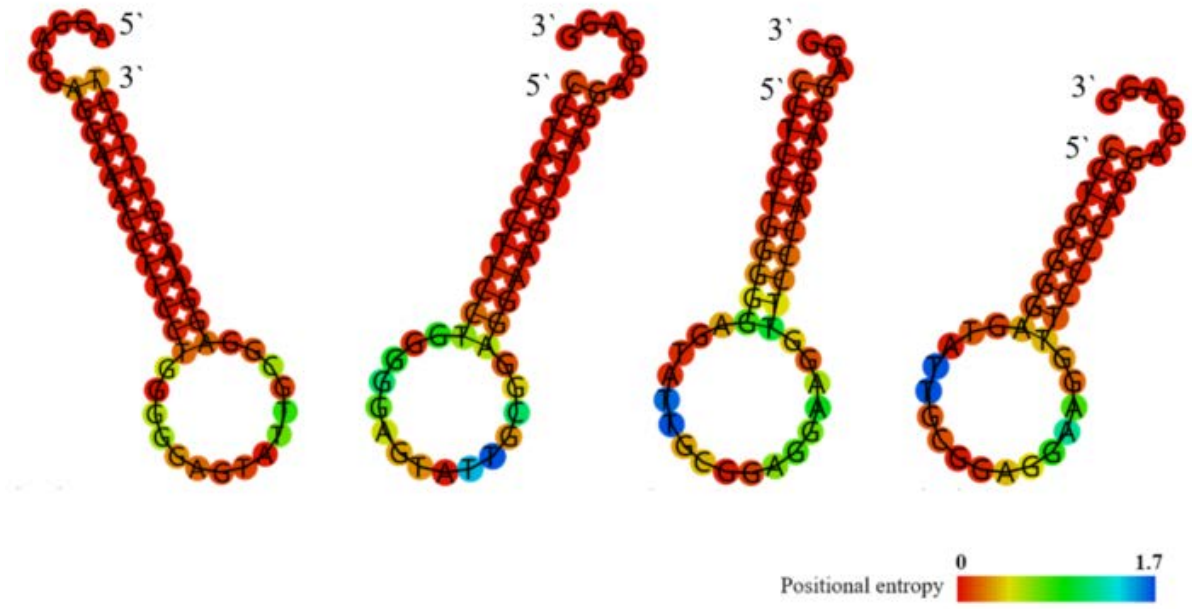


**Figure S6.** The prediction for hairpin structures of the seizes of AMP-TG-hairpin sequences by the RNAfold20 program (at http://rna.tbi.univie.ac.at/)

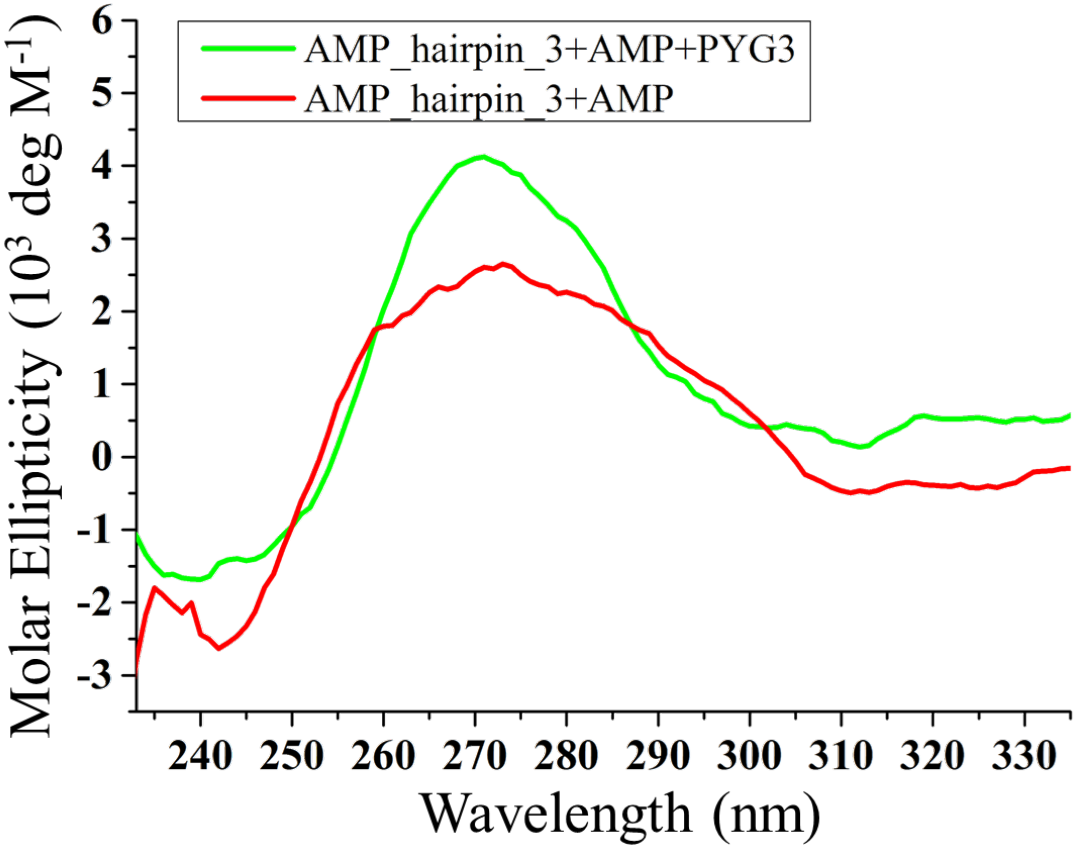


**Figure S7.** The circular dichroism spectra of bio-sensor (10 μM) system with/without AMP (20 μM) in $Na^+/K^+$ buffer

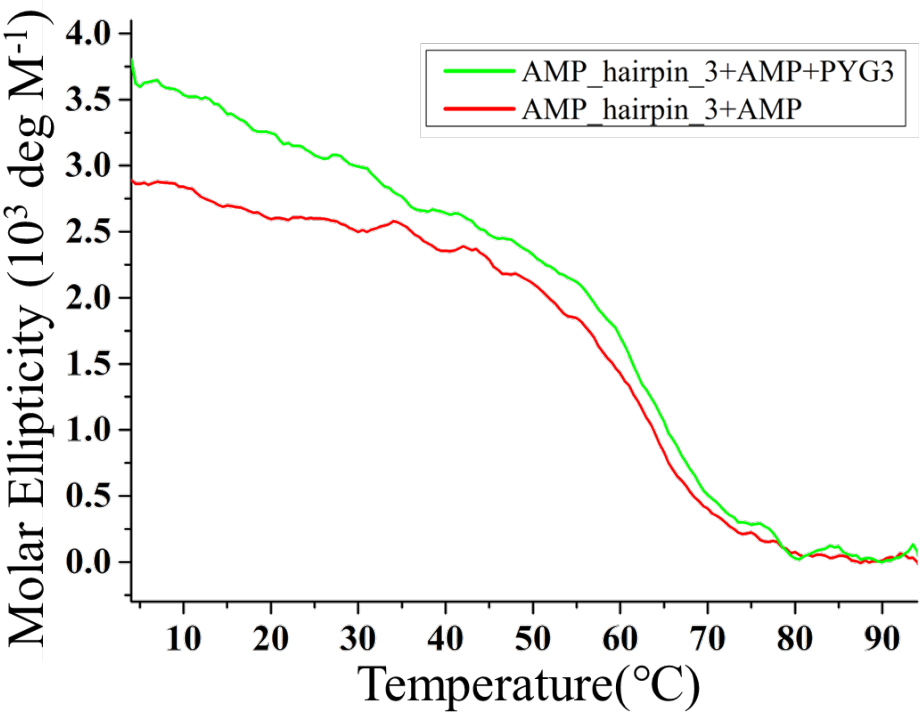


**Figure S8.** The melting curves of bio-sensor (10 μM) system with/without AMP (20 μM) in $Na^+/K^+$ buffer

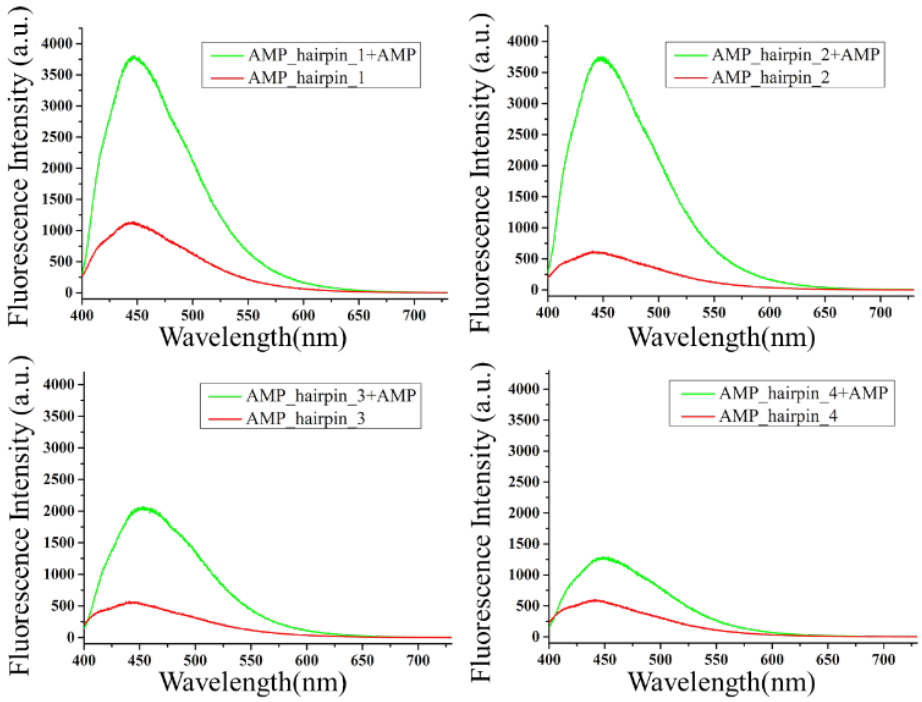


**Figure S9.** Fluorescence spectra from biosensor systems in response to AMP. Each biosensor system contained AMP-TG-hairpin (1 μM) and PYG3 (1 μM) with/without AMP (5 mM) in a $Na^+/K^+$ solution (NaCl 100 mM, KCl 10 mM).

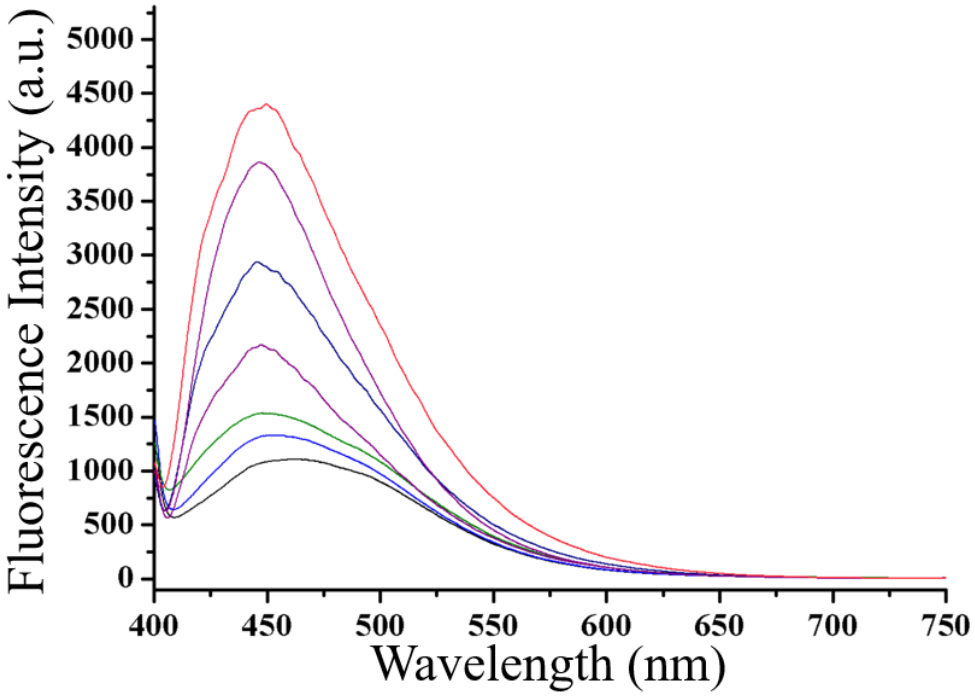


**Figure S10.** Fluorescence spectra from biosensor systems based on AMP-hairpin_2 (1 μM) and PYG3 (1 μM) in response to AMP (0 mM, 0.25 mM, 0.5 mM, 1 mM, 2.5 mM, 5mM, 10 mM).

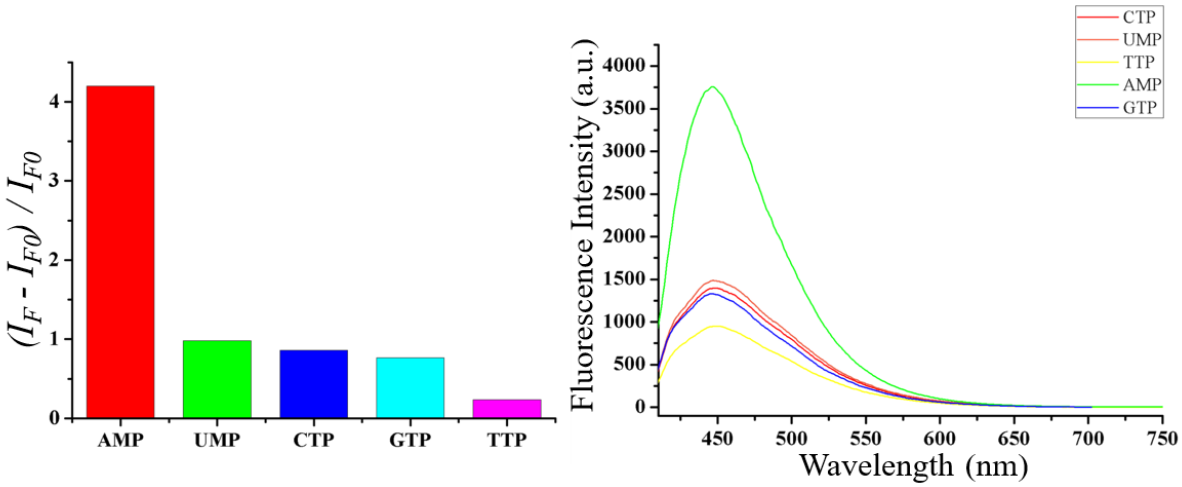


**Figure S11.** Sensor AMP selectivity against AMP analogues, UMP, CTP, GTP, TTP (5 mM) in the $Na^+/K^+$ solution.

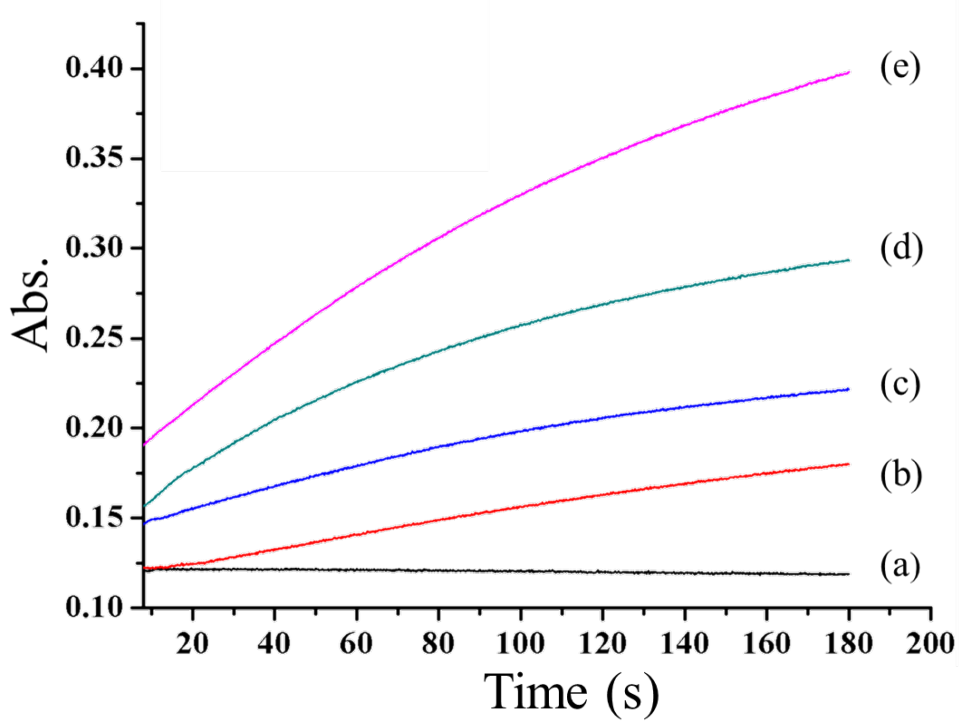


**Figure S12.** Time-dependent absorption upon analyzing AMP by the bio-sensor based on aptamer-DNAzyme hairpin which was reported by Itamar Willner's group. (a) 0 M, (b) 50 μM, (c) 500 μM, (d) 2.5 mM, and (e) 5 mM.

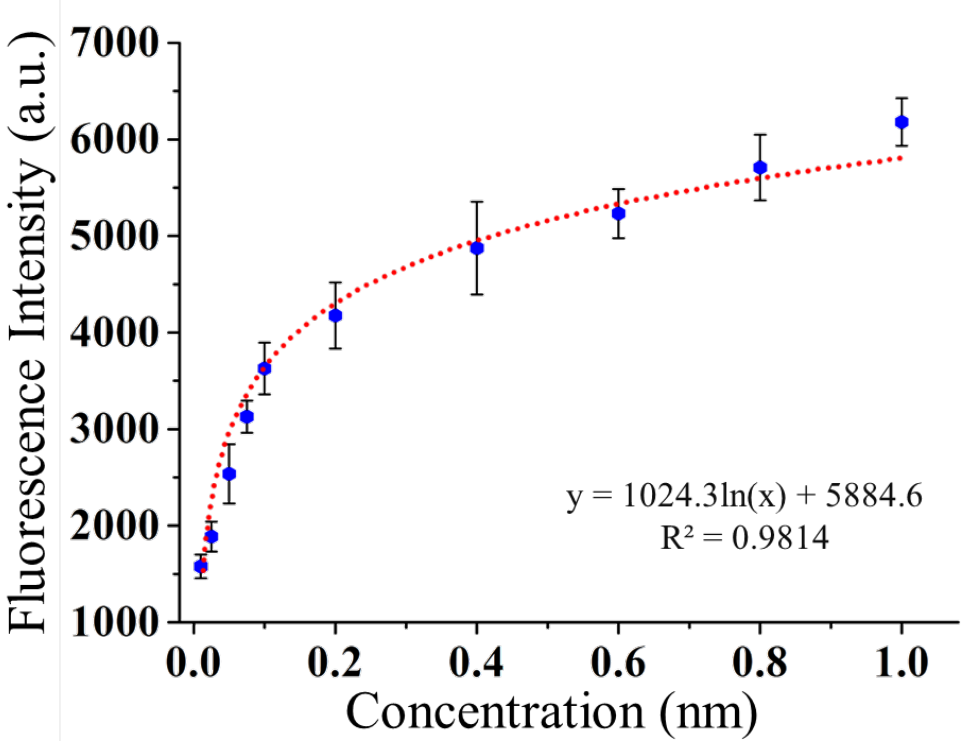


**Figure S13.** Logistic fitting of the fluorescence intensity and PYG3 (1 μM) with different concentrations of TG-AS1411 (10 nM, 25 nM, 50 nM, 75 nm, 0.1 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, 1 μM)