Supplementary Material (ESI) for Chemical Communications
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Label Free Visual Detection of Nucleic Acid in Biological Samples with Single Base Mismatch Detection Capability

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Chemicals and Reagents

All probes used are listed in Table S1 and were synthesized in house on a PolyGen DNA synthesizer. DNA synthesis reagents were purchased from Glen Research (VA, USA). Agilent 1100 HPLC with a Promosil C18 reversed phase column (5 μm, 250×4.6 nm) was used for probe purification. Absorbance was measured on an Agilent 8453 UV/Vis spectrometer for concentration calculation. Streptavidin beads were obtained from GE Healthcare (NJ, USA). Hemin and ABTS were purchased from Sigma (Shanghai, China).

Table S1. DNA sequences of probes used in this work

Name	Sequence
β-Actin-aMB	ATTGACCGCTGTGTGACGCAACACTCAAT***TTCTCCAGTGTAGTATTAGG CAATG***AAATTGAGTGTT
Flag-aMB	ATTGACCGCTGTGTGACGCAACACTCAAT***GCCCGGGCTCTTATCGTCGTC AT***GGGCATTGAGTGT
SNP-aMB	ATTGACCGCTGTGTGACGCAACACTCAAT***TCTGGTCTGAAGGTTTAT T***GAATTGAGTGTT
SNP-cDNA	AAT AAA CCT TCA GAC
G-quartet probe	AGGGAGGGAGGGAGGGTCAACACTCAAT

Visual Detection Experiment

First, 500nM aMB probe and target DNA/RNA were incubated in 200μL Tris-HCl buffer system (25mM Tris-HCl, 120mM NaCl, 5mM KCl, pH 7.4) for 10 minutes at 95°C, and then annealed to room temperature in about 25minutes. Secondly, 5μL Streptavidin beads(7.5×10^4) were added and incubated for 45 minutes at room temperature. After removing the solution by the tips with filter, the beads were

washed with 3×100μL Tris-HCl buffer. Then, the beads were transferred to 200μL Tris-HCl buffer containing 2μM G-quartet Probe, and incubated for 30 minutes at room temperature. The beads was washed and transferred to 20μL Tris-HCl buffer system containing 2μL 10μM hemin, 0.8μL 50mM ABTS, 1.4μL 58mM H_2O_2 , and 15.8μL Tris-HCl buffer. Hemin was added ahead of ABTS and H_2O_2 about 20 minutes, in order to stabilize the structure of G-quartet and promote the interaction between them. About 15 minutes later, a color change phenomenon can be observed in solution.

β-Actin-aMB Design

Table S2. DNA sequences of TMR-labeled β-Actin-aMB

Name	Sequence
β-Actin-aMB1	ATTGACCGCTGTGTGACG CAACACTCAAT***AGGAAGGAAGGCTGG AAG AG*** CCTATT GAGTGT -TMR
β-Actin-aMB2	ATTGACCGCTGTGTGACG CAACACTCAAT***AGGAAGGAAGGCTGG AAG AG***CCTATTGAGT-TMR
β-Actin-aMB3	ATTGACCGCTGTGTGACG CAACACTCAAT***AGGAAGGAAGGCTGG AAG AG*** TCCT ATT GAG TGTT-TMR

Underline indicates the stem.

These three probes have different lengths of stem, tested by flow cytometry to find the one performing best.

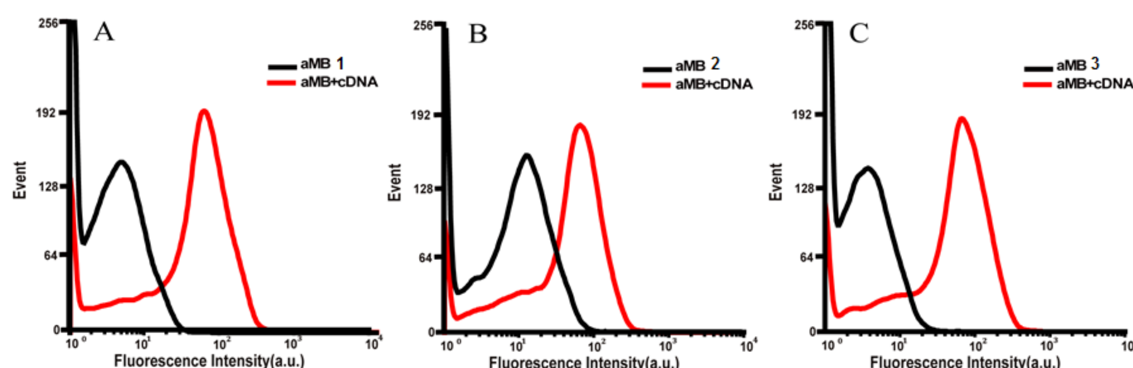

Fig. S1 Flow cytometry analysis of 500nM aMB and 500nM aMB+2.5μM cDNA

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HEK-293FT PEI transfection

HEK-293 FT cells were splitted one day before transfection in DMEM/10% FBS medium (10cm dish: 4.0×10^6 cells). Prior to transfection, all reagents were brought to room temperature. Total plasmid DNA (ug) were diluted in a sterile tube with serum-free DMEM w/o phenol red (volume of media is 10% of final volume in culture vessel). The transgene was prepared by mixing viral packaging (psPAX2), viral envelope (pMD2G) and DNA at 4:2:1 ratio. For 10cm dish, the total volume is 1mL with 7-8 ug of total DNA. PEI (1ug/uL) was added to the diluted DNA, mixed immediately by vortexing or pipeting, and incubated at room temperature for 15 minutes. The volume of PEI used is based on a 3:1 ratio of PEI (ug) : total DNA(ug). For 10cm dish, 21ul of PEI (1ug/uL), that is 21ug, was added. Then the mixture was added to cells. After 24 hours post-transfection, the transfected cells were harvested.

Western Blot

Equal amounts of protein lysate were subjected to SDS–PAGE and transferred onto nitrocellulose membranes. Blogs were probed with the specific primary antibodies. Antibodies used for Western blog were as follows: antibodies against Flag (Sigma), beta-actin (Sigma). After extensive washing, blots were then incubated with horseradish peroxidase conjugated secondary antibody.