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Catalytic activity of dual-hemin labelled oligonucleotide: conformational dependence and fluorescent DNA sensing†

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Experimental

Materials and reagents. All oligonucleotides were synthesized and purified using high-performance liquid chromatography by TAKARA Biotechnology (Dalian, China), and their sequences were listed as follows:

Probe: 5'-hemin-GTGTGAGTCTGTGTGTGTTTTT-hemin-3'

Target: 5'-ACACACACAGACTCACAC-3'

Single-base mismatched target (smT): 5'-ACACACACA*C*ACTCACAC-3'

Three-base mismatched target (tmT): 5'-ACACA*G*ACA*C*ACT*G*ACAC-3'

Non-complementary target (ncT): 5'-AACTCATGTTCAAGACAG-3'

Reference probe 1 (rP1): 5'-hemin-CTGACGAGTCCTTCCACGATACCAGTCAG-hemin-3'

Reference probe 2 (rP2): 5'-ROX-GTGTGAGTCTGTGTGTGTTTTT-hemin-3'

Here, mismatched bases are highlighted in blue italic type. The probe and reference probe 1 (rP1) were labeled with two hemin molecules at both 5' and 3' ends, while the reference probe 2 (rP2) was labeled with X-rhodamine (ROX) and hemin at 5' and 3' ends, respectively. The probes were all characterized with mass spectroscopy (Fig. S1-S3).

Tyramine and tris(hydroxymethyl) aminomethane (tris) were purchased from Sigma–Aldrich Inc (USA). Tris−HCl buffer (50 mM, containing 100 mM NaCl, pH 7.4) was used for homogeneous fluorescence measurements. All other reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays.

Apparatus. Fluorescence spectra were recorded on a RF-5301PC spectrofluorometer (Shimadzu, Japan) equipped with a xenon lamp. To measure fluorescent signal of dityramine and ROX, the excitation wavelengths were set to 320 nm and 570 nm, respectively, and the excitation and emission slits were both set to 10 nm. Mass spectra were recorded on an Autoflex III Smartbeam mass spectrometer (Bruker, Germany).

Homogenous Fluorescent Detection of DNA. After 198 μL mixture of probe (10.1 nM) and target DNA was incubated in 50 mM Tris-HCl buffer (pH 7.4, containing 100 mM NaCl) at room temperature for 10 min, 1.0 μL tyramine (140 mM) and 1.0 μL H_2O_2 (400 mM) were added to initiate the catalytic reaction for 10 min. Finally, the fluorescence spectrum was recorded at an excitation wavelength of 320 nm.

Mass spectra of hemin labelled probes

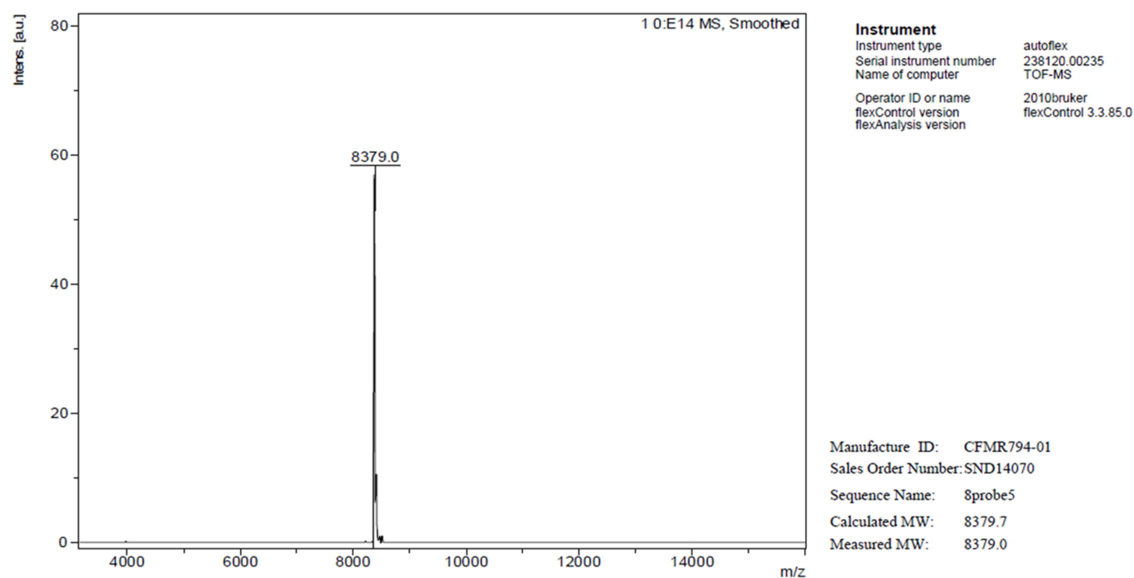


Fig. S1 Mass spectrum of probe. Calculated MW: 8379.7, measured MW: 8379.0.

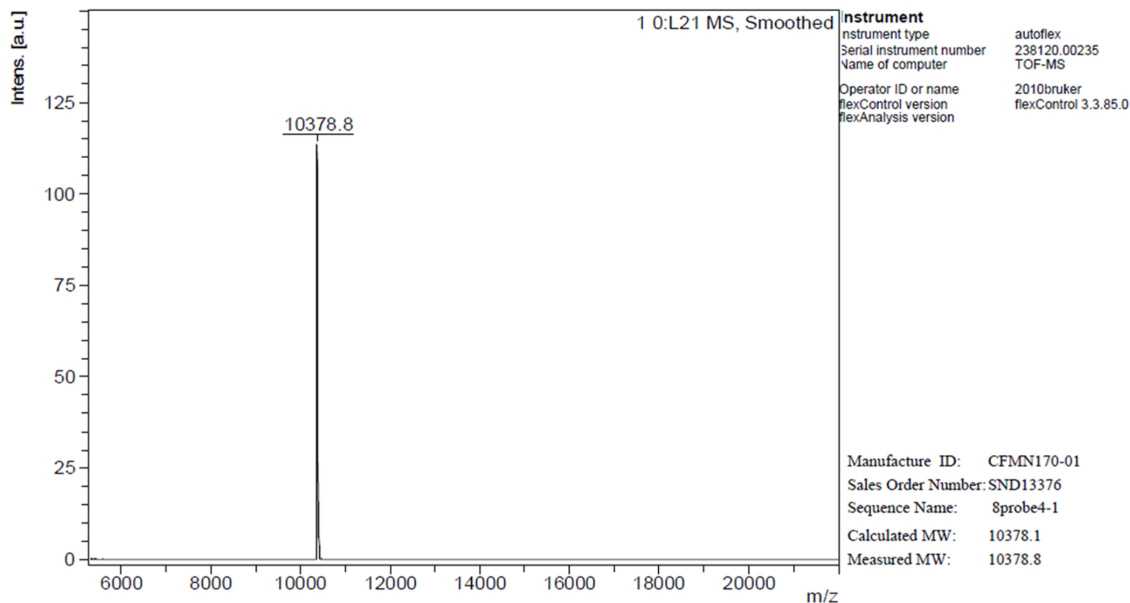


Fig. S2 Mass spectrum of rP1. Calculated MW: 10378.1, measured MW: 10378.8.

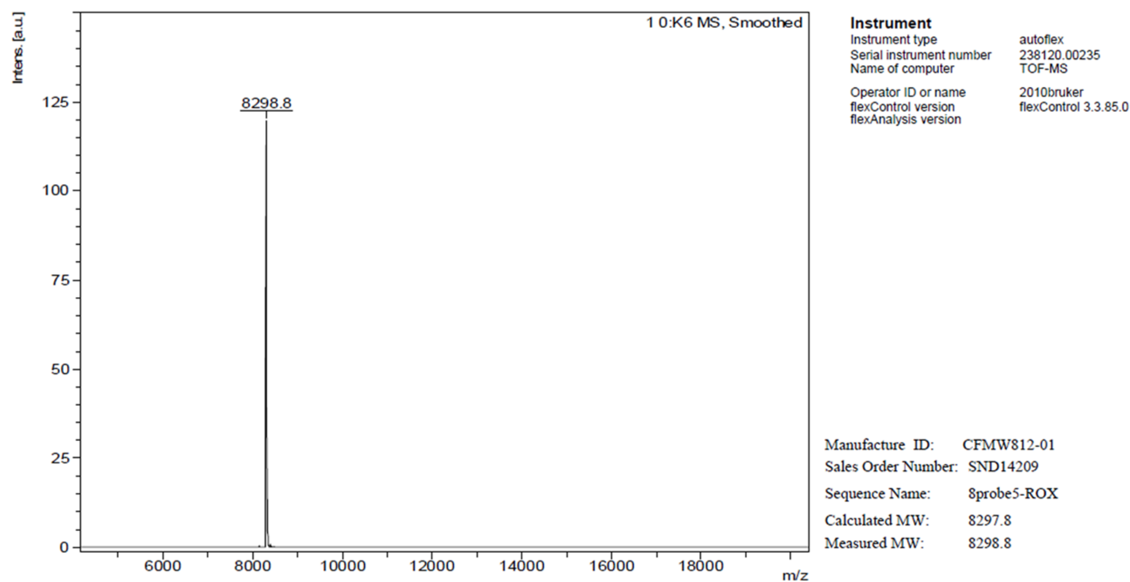


Fig. S3 Mass spectrum of rP2. Calculated MW: 8297.8, measured MW: 8298.8.

UV-visible absorption spectrum of rP1

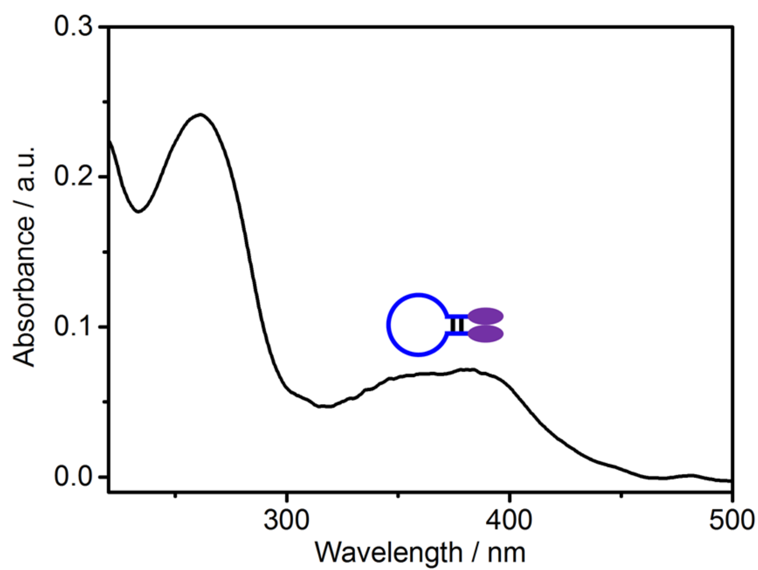


Fig. S4 UV-visible absorption spectrum of 1 μM rP1.

Optimization for homogenous fluorescence DNA sensing

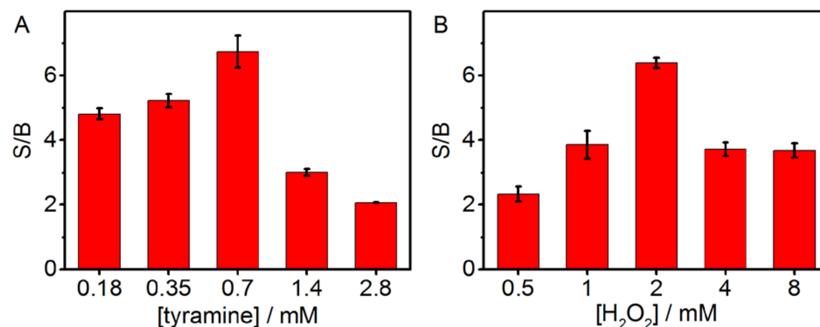


Fig. S5 Effects of (A) tyramine and (B) H_2O_2 concentration on S/B, where S and B are fluorescent signals in presence of 10 nM probe/target duplex and probe, respectively.

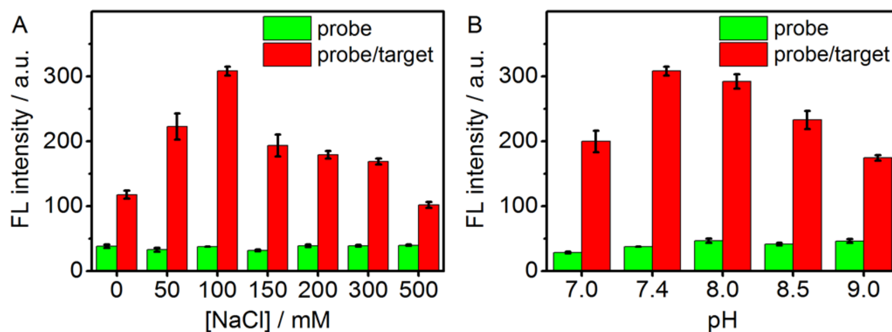
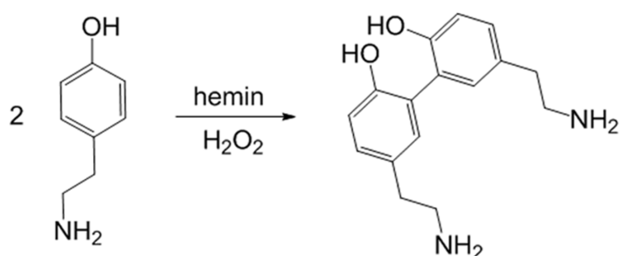


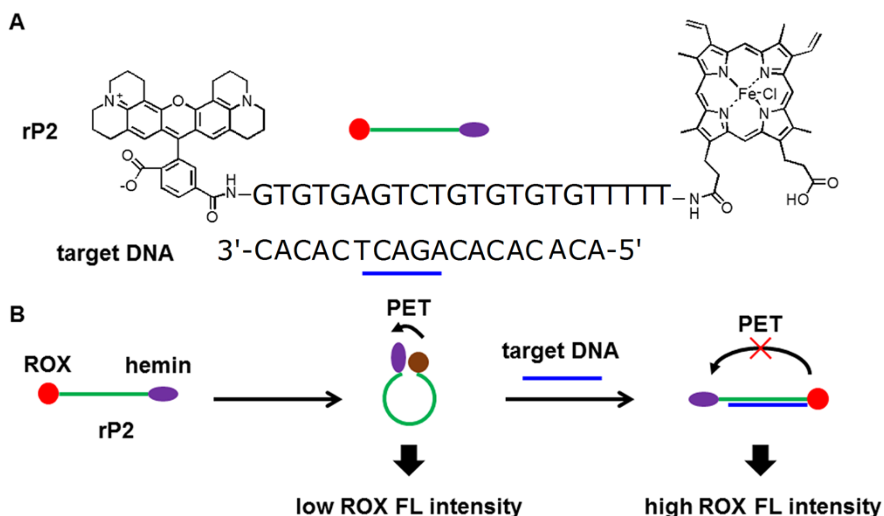
Fig. S6 Effects of (A) NaCl concentration and (B) pH of reaction buffer on fluorescent signal of dityramine formed in presence of 10 nM probe (green) and probe/target duplex (red).

Catalytic reaction of hemin



Scheme S1 Catalytic oxidation reaction of non-fluorescent tyramine in the presence of hemin and H_2O_2 to form fluorescent dityramine.

Target DNA regulated dimer dissociation in rP2



Scheme S2 Schematic illustration of (A) chemical structure of rP2, and (B) target DNA regulated dimer dissociation of hemin and ROX.

Comparison among analytical properties of dual-hemin labeled probe, molecular beacon and G-quadruplex/hemin DNAzyme

Although the preparation of both dual-hemin probe and molecular beacon need a labeling step, they are ready-to-use probes, while hemin-DNAzyme is only a signal reporter without a specific recognition unit.[S1] Thus, hemin-DNAzyme must be labeled to recognition unit for its utlization. The ready-to-use probes can shorten the detection time. Compared with molecular beacon, which outputs the detection signal in a "one target to one signal molecule" model, both dual-hemin labeled probe and hemin-DNAzyme can output the detection signal in a "one target to multiple signal molecules" model due to their peroxidase activity, thus they can improve the detection sensitivity.

Supporting references

S1. (a) V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler and I. Willner, *Anal. Chem.*, 2004, **76**, 2152-2156; (b) E. Golub, R. Freeman, A. Niazov and I. Willner, *Analyst*, 2011, **136**, 4397-4401; (c) Y. Xiao, V. Pavlov, T. Niazov, A. Dishon, M. Kotler and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 7430-7431.