Electronic Supplementary Information (ESI)

Superquenched DNAzyme-perylene complex: a convenient, universal and low-background strategy for fluorescence catalytic biosensors

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Table of contents

1. Experimental section

Reagents and Apparatus

Perylenetetracarboxylic dianhydride and N, N′-Dimethyl-1, 3-propanediamine was obtained from Alfa Aesar. Methyl iodide, L-hitidine and other compounds were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). All chemicals were of analytical grade unless otherwise specified. Triply distilled water (resistance >18 MΩ.cm) was used for preparation of all solutions by a Milli-Q system (Millipore, USA).

Oligonucleotides designed in this study were synthesized by Takara Biotechnology (Dalian, China) and used without further purification. The DNA sequences are as follows:

DS: 5′-FAM-CACTrAGGAAGAGATGATT-3′

GR-5 DNAzyme: 5′-AATCATCTCTGAAGTAGCGCCGCCGTAGTG-3′

HD3 DNAzyme: 5′-AAT CAT CTCT T AAC GGG GCT GTG CGG CTA GGA AGT A AGTG-3′

Fluorescence spectra were measured using a fluoromax-4 spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ) with both excitation and emission slit set at 5.0 nm. A quartz fluorescence cell with an optical path length of 1.0 cm was used. Excitation wavelength was excited at 494 nm, and the emission spectra from 505 to 600 nm were collected. All measurements were carried out at room temperature unless stated otherwise. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

Synthesis of compound 1

Cationic perylene diimide derivative **1** was synthesized following the literature procedure.[S1] Briefly, under nitrogen atmosphere, a mixture of perylene tetracarboxylic dianhydride (1.0 g, 2.42 mmol) and N, N′-Dimethyl-1, 3-propanediamine (5 mL, 39.3 mmol) in 50 mL of isobutanol was stirred at 90 °C overnight. After filtering, the solid was washed twice with water and ethanol. The unreacted perylene tetracarboxylic dianhydride was then removed from the crude product by treating with 5% aqueous

NaOH solution at 90 °C for 1 h. After filtering, washing with triply distilled water and ethanol, and drying under vacuum, 1.1g of N, N′-bis(propylenedimethylamine)-3, 4: 9, 10-perylenediimide was obtained as a red solid. It was added together with 1.5 mL of methyl iodide (24.1 mmol) to 50 mL of toluene, and heat to reflux for 3 h under nitrogen. The mixture was slowly brought to room temperature, and then filtered and washed with cool ether to give an iodide of compound **1** as a brown-red solid. The nitrate of compound **1** was further prepared by treating it with silver nitrate. It was characterized using NMR and MS analytical spectroscopic techniques (data not shown), agreeing well with the previously reported data. [S1]

Procedure for fluorescence catalytic detection

For fluorescence catalytic detection of Pb^{2+}, the GR-5 DNAzyme strand (30 nM) was first incubated with the substrate strand (50 nM) in a buffer solution (50 mM HEPES, 50 mM NaCl, 5 mM $MgCl_2$, pH 7.4) at room temperature for 20 min to allow the oligonucleotides efficiently hybridized. After the addition of varying concentrations of Pb^{2+} into the mixture and incubating for another 20 min, the resulting solution was then mixed with 1.4 μM (final concentration) of compound **1** for 5 min prior to detection. Control experiment for the fluorescence biosensors in the absence of Pb^{2+} was also carried out following the procedure shown above. The FluoroMax-4 spectrofluorometer was used to record the fluorescence spectra from 505 to 600 nm upon excitation at 494 nm. The experiments were performed in 100 μL solution.

To detect L-histidine with the fluorescence catalytic biosensor, 30 nM of the L-histidine-dependent DNAzyme strand was first incubated with 50 nM of the FAM-labeled substrate strand at room temperature for 20 min in a buffer solution containing 25 mM HEPES (pH 7.4) and 500 mM KCl. Same procedure as that of detection of Pb^{2+} was then performed except using different concentrations of L-histidine to replace Pb^{2+} to initiate the cleavage reaction.

2. Supplementary Figures and Table

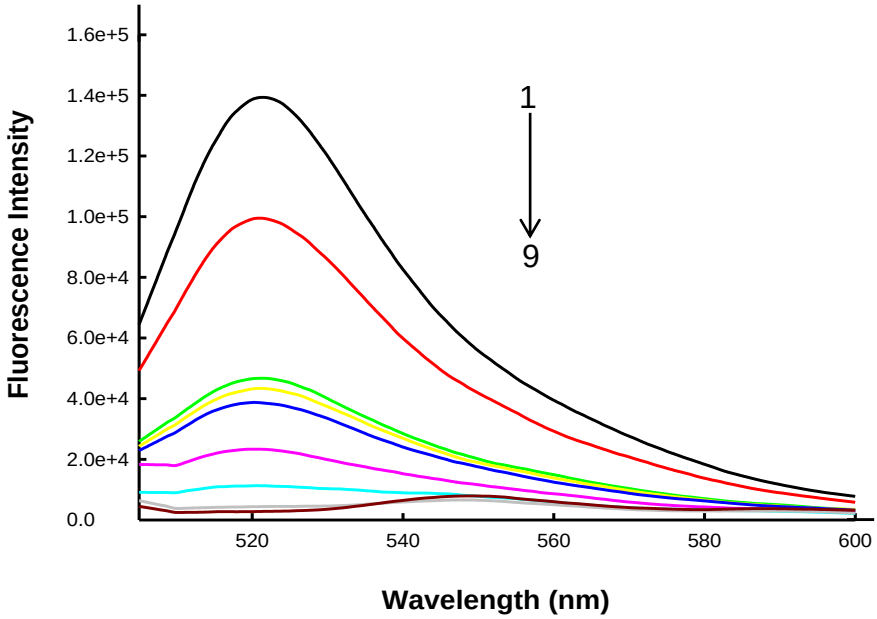

Fig. S1 The effect of different compound 1 concentration on the fluorescence response of the sensing system: (1) 0nM; (2)160nM; (3)300nM; (4) 500nM; (5)700nM; (6)900nM; (7)1100nM; (8)1400nM; (9)1700nM. The concentration of DNAzyme and DS is 30nM and 50nM, respectively.

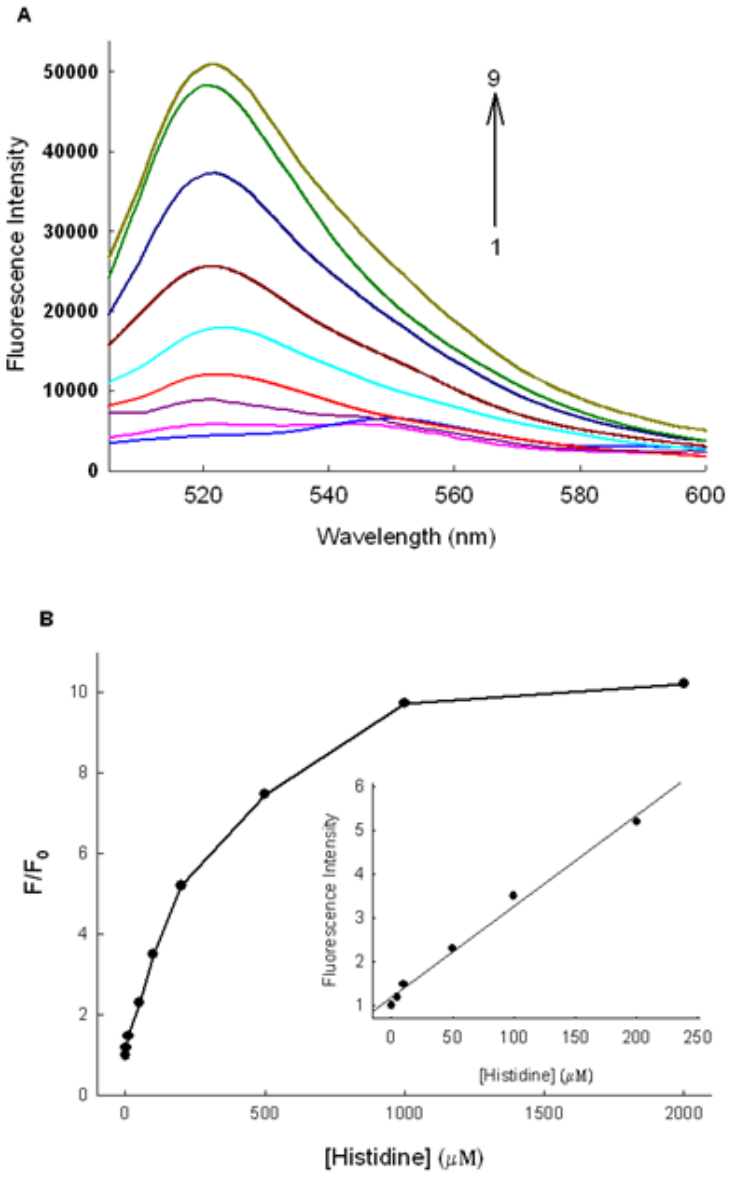

Fig. S2 (a) Fluorescence emission spectra of the sensing system on exposure to L-histidine solutions of different concentrations : (1) 0; (2) 2 μM; (3) 10 μM; (4) 50 μM; (5) 100 μM; (6) 200 μM; (7) 500 μM; (8) 1 mM; (9) 2 mM; (b) Calibration curve of the sensing system for Pb2+. The curve was plotted with the fluorescence enhancement vs. L-histidine concentration. Inset shows the linear responses at low L-histidine concentrations.

Table S1. Recovery experiments of Pb^{2+} in river water samples

River water	Pb^{2+} spiked (nM)	Pb^{2+} recovered (nM)	Recovery (%)
1	10.0	9.4[a]±0.4[b]	94.0
2	20.0	22.0[a]±2.6[b]	110.0
3	100.0	102.4[a]±4.5[b]	102.4

[a] Mean values of four determinations. [b] Standard deviation.

3. References

S1. B. Wang, H. Jiao, W. Li, D. Liao, F. Wang and C. Yu, *Chem. Commun.*, 2011, **47**, 10269.