

**A novel fluorescence strategy for mercury ions and trypsin activity assay based on nitrogen-doped graphene quantum dots**

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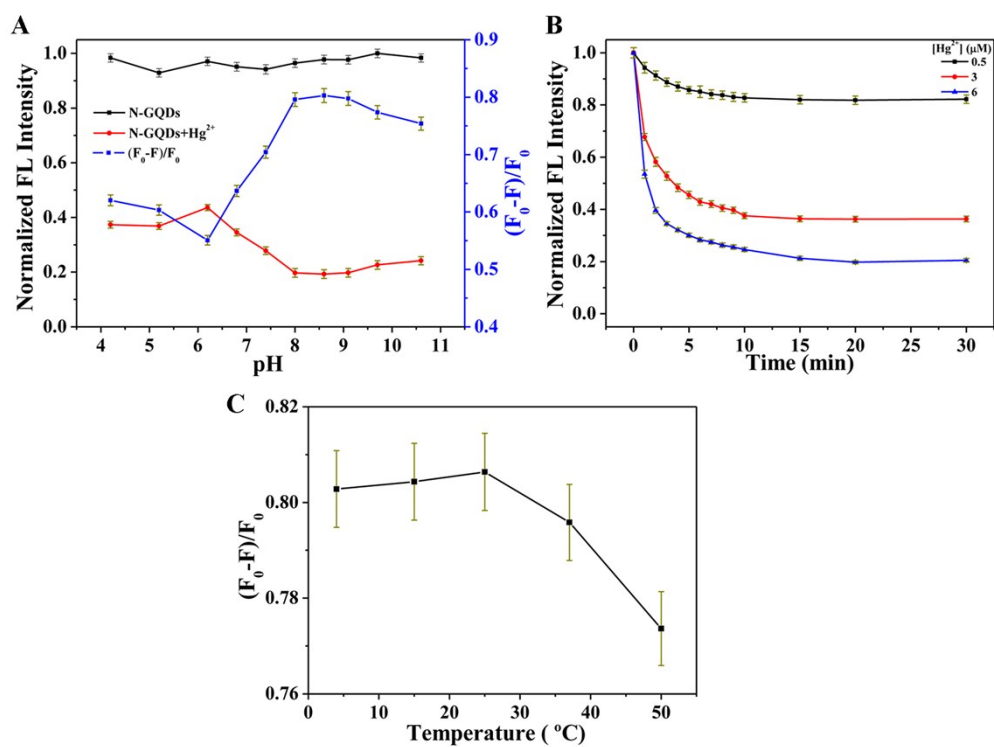
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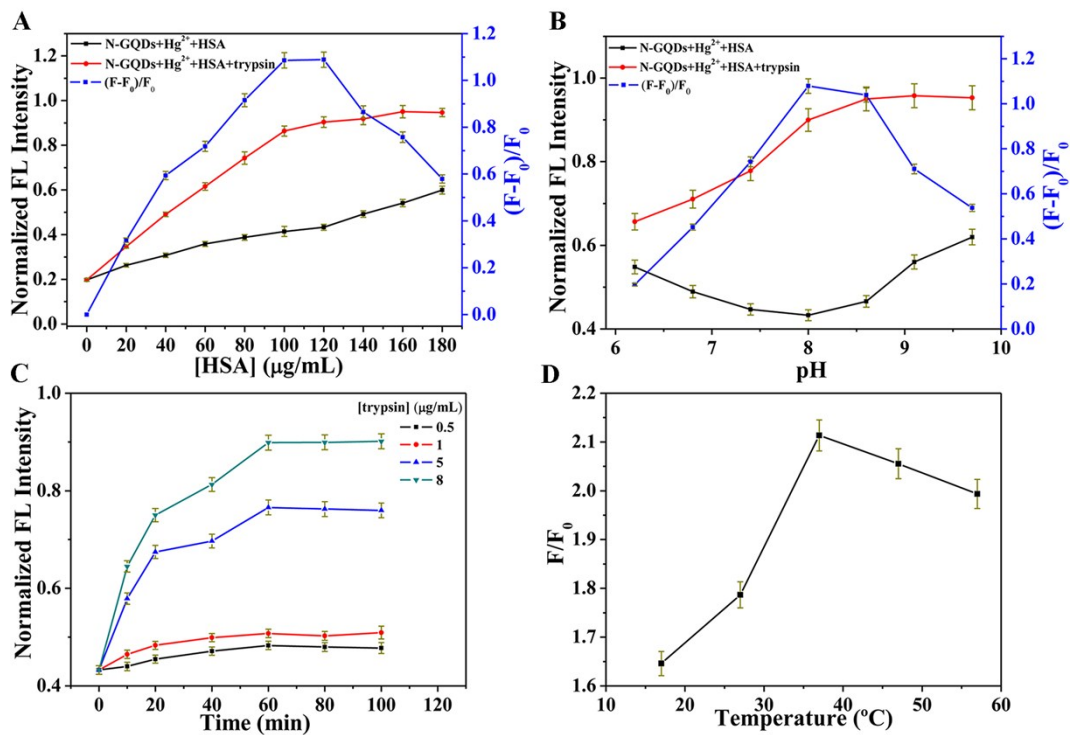
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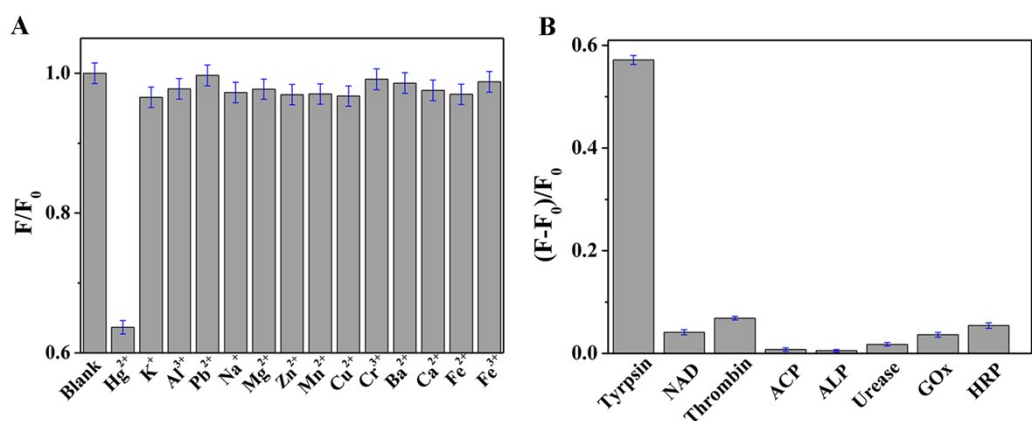
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**Fig. S1** (A) The effect of pH in the range of 4.2-10.6 on Hg<sup>2+</sup> assay; (B) The effect of reaction time in the presence of different concentrations of Hg<sup>2+</sup> on Hg<sup>2+</sup> assay; (C) The effect of reaction temperature in the range of 4-50 °C on Hg<sup>2+</sup> assay.



**Fig. S2** (A) The effect of the concentration of HSA on trypsin activity assay; (B) The effect of the pH on trypsin activity assay; (C) The effect of the enzymatic incubation time in the presence of different concentrations of trypsin on trypsin activity assay; (D) The effect of the enzymatic incubation temperature on trypsin activity assay.



**Fig. S3** (A) The selectivity of N-GQDs for Hg<sup>2+</sup> assay. The Hg<sup>2+</sup> concentration was 1  $\mu$ M while metal ions (Al<sup>3+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, Cr<sup>3+</sup>, Ba<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>) were 10  $\mu$ M and metal ions (K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>) were 10 mM; (B) The selectivity of the proposed nanosensor for trypsin activity assay. The trypsin concentration was 4  $\mu$ g/mL while  $\beta$ -nicotinamide adenine dinucleotide (NAD), thrombin, horse radish peroxidase (HRP) were four times (16  $\mu$ g/mL ) and acid phosphatase (ACP), alkaline phosphatase (ALP), urease, glucose oxidase (GOx) were ten times (40  $\mu$ g/mL ). Conditions: [Hg<sup>2+</sup>] = 6  $\mu$ M, [HSA] = 120  $\mu$ g/mL, pH = 8.0. The error bar represented standard deviation of three replicate measurements.

**Table S1** Comparison of different methods for the determination of Hg<sup>2+</sup>.

Methods	Probe	Linear range (μM)	LOD (μM)	References
Fluorescence	CuDTC2-CDs	0.04-1	0.02	[1]
Fluorescence	N-CDs	0-24	0.02	[2]
Fluorescence	CDs	0.1-20	0.062	[3]
Fluorescence	N. B-CDs	0.02-0.16	0.0073	[4]
Fluorescence	CDs-labeled DNA	0.005-0.2	0.0026	[5]
Colorimetry	L-Cys-GNPs	0.1-2	0.1	[6]
Fluorescence	N-GQDs	0.02-1	0.0047	This work

**Table S2** Comparison of different methods for the determination of trypsin.

Methods	System	Linear range ( $\mu\text{g/mL}$ )	LOD ( $\mu\text{g/mL}$ )	References
Fluorescence	BSA-GQDs/CMR2	0-6	0.7	[7]
Fluorescence	Mn ZnSe QDs/Arg6	0.1-12	0.04	[8]
Fluorescence	GO/Arg6-FAM	0.1-10	0.1	[9]
Fluorescence	Conjugated polymers (F-BSF)	0-2.5	0.2	[10]
Fluorescence	Polymer/BSA/Cu <sup>2+</sup>	0-8	0.0525	[11]
Colorimetry	BSA-AuNCs/TMB	0.9-1000	0.6	[12]
Colorimetry	H <sub>2</sub> O <sub>2</sub> /cyt c/TMB	0.05-1	0.0045	[13]
Electrochemistry	electrochemical probes	0.005-0.15	0.0018	[14]
Fluorescence	N-GQDs/Hg <sup>2+</sup> /HSA	0.03-8	0.0063	This work

**Table S3** Determination of Hg<sup>2+</sup> in real water samples.

Samples	Added ( $\mu\text{M}$ )	founded ( $\mu\text{M}$ )	Recovery (%)	RSD (n = 3, %)
Tap water	0	-	-	-
	0.08	0.07	87.5	1.01
	0.40	0.41	102.5	2.69
	0.90	0.87	96.7	1.67
Lake water	0	-	-	-
	0.08	0.08	100.0	1.06
	0.40	0.37	92.5	1.70
	0.90	0.85	94.4	1.14

**Table S4** Determination of trypsin in human serum samples.

Samples	Added ( $\mu\text{M}$ )	Founded ( $\mu\text{M}$ )	Recovery (%)	RSD (n = 3, %)
1	0	-	-	-
	0.50	0.55	110.0	0.80
	5.00	5.09	101.8	0.70
	7.00	6.92	98.9	0.71
2	0	-	-	-
	0.50	0.52	104.0	1.35
	5.00	5.14	102.8	1.86
	7.00	7.16	102.3	0.61



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