Electronic Supplementary Information

For

FRET-enhanced nanoflares for sensitive and rapid detection of ampicillin

Wenjing Wang, Wenyi Lv, Ziyu Pan, Lei Zhan* and Chengzhi Huang*

Key Laboratory of Luminescent and Real-Time Analytical System (Southwest University), Chongqing Science and Technology Bureau, College of Pharmaceutical Science, Southwest University, Chongqing 400715, China.

E-mail: chengzhi@swu.edu.cn (Chengzhi. Huang);

zhanlei6@swu.edu.cn (Lei. Zhan),

Table S1. DNA sequences used in the experiment.

Entry	Sequence (5′ - 3′)
Aptamer	SH-TTT TTT TTTT TGG GGG TTG AGG CTA AGC CGA C
cDNA-1	AAC CCC CAT T-TAMRA
cDNA-2	TCA ACC CCC ATT-TAMRA
cDNA-3	CCT CAA CCC CCA TT-TAMRA
cDNA-4	AGC CTC AAC CCC CAT T-TAMRA

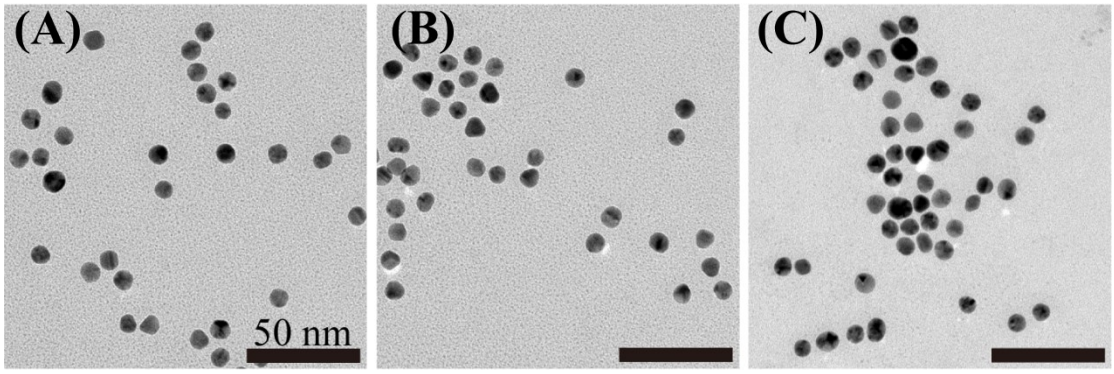

Fig. S1. TEM images of the prepared AuNPs (A), nanoflares (B) and the enhanced nanoflares (C).

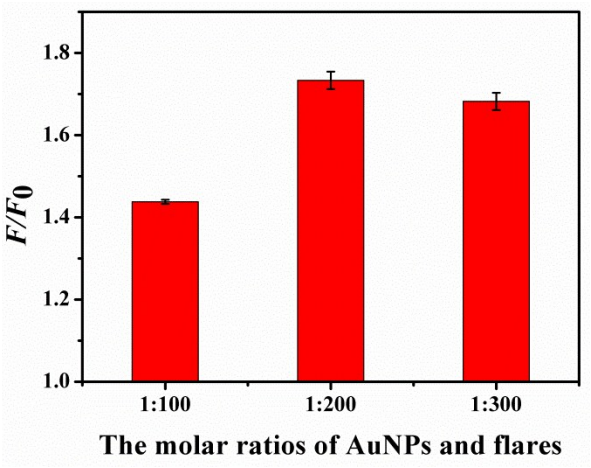

Fig. S2. The fluorescence recovery of the enhanced nanoflares after reaction with AMP in the different molar ratios of AuNPs and the flares. In the optimization experiment, the concentration of AMP was 13.5 ng/mL. 50 μL of AMP was added into 200 μL enhanced nanoflares and reacted in PBS buffer at 25 °C for 1h. F and F_0 represented the fluorescence intensity of the enhanced nanoflares in the presence and absence of AMP, respectively.

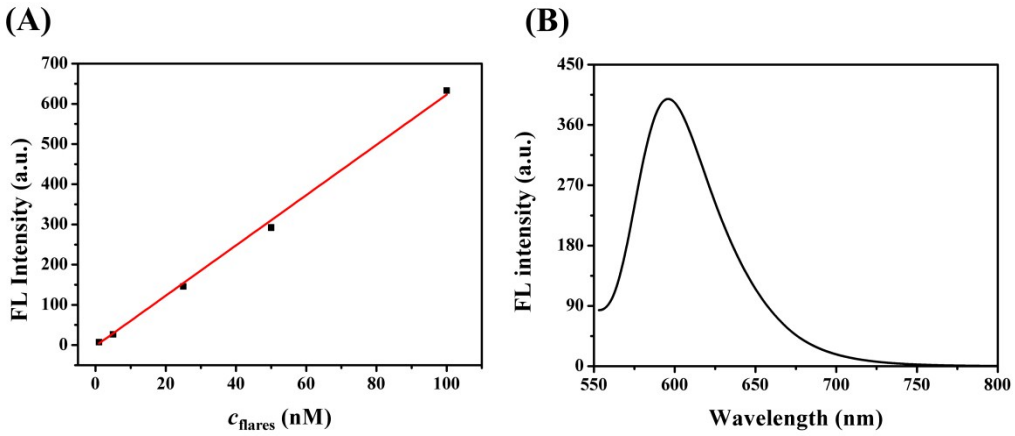

Fig. S3. Evaluation of amounts of the flares on each AuNP. (A) Standard linear calibration curve of fluorescence intensity against the concentration of TAMRA-labelled flares. (B) The fluorescence spectrum of supernatant containing the flares replaced by β-mercaptoethanol. The excitation wavelength was 543 nm and the emission wavelength was from 553 to 800 nm.

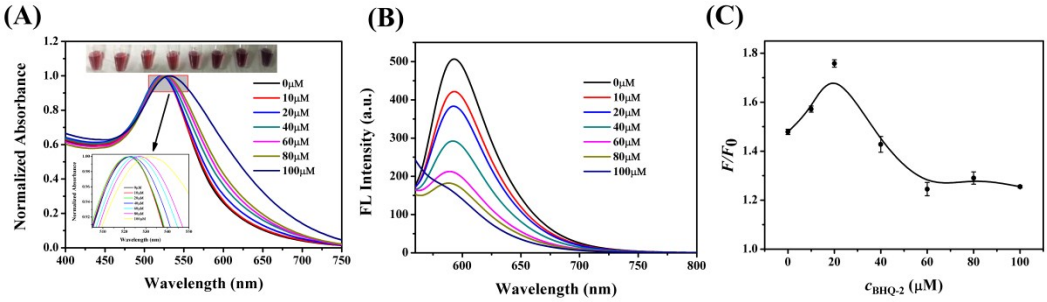

Fig. S4. UV−vis spectra (A) and fluorescence spectra (B) of the enhanced nanoflares in different concentrations of BHQ-2. Inset in top-left of (A): photograph of the enhanced nanoflares with increase the concentration of BHQ-2. Inset in bottom-left of (A): partial enlargement of the UV−vis spectra of the enhanced nanoflares with different concentration BHQ-2. (C) The fluorescence recovery of the prepared enhanced nanoflares after reaction with AMP in different concentration BHQ-2. In the optimization experiment, the concentration of AMP was 13.5 ng/mL. 50 μL of AMP was added into 200 μL enhanced nanoflares and reacted in PBS buffer at 25 °C for 1h. F and F_0 represented the fluorescence intensity of the enhanced nanoflares in the presence and absence of AMP, respectively.

Table S2. Fluorescence lifetimes obtained with biexponential fit of the fluorescence decay curves of the flare, nanoflare and the enhanced nanoflare.

Samples	τ_1 [ns] (%)	τ_2 [ns] (%)	τ_{ave} [ns] (%)
Flares	3.9 (54.12)	5.6 (45.88)	4.7
Nanoflares	3.2 (47.17)	4.4 (52.83)	3.8
Enhanced nanoflares	3.2 (57.95)	4.5 (42.05)	3.7

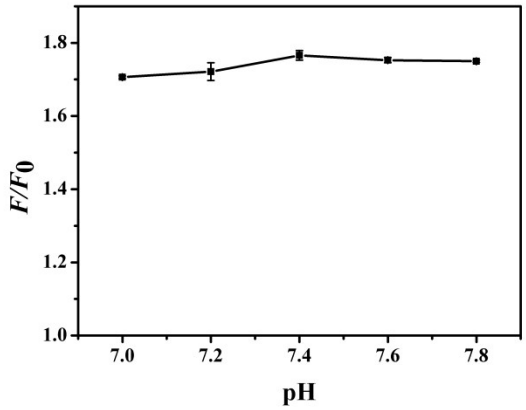

Fig. S5. The fluorescence recovery of the enhanced nanoflares after reaction with AMP in different pH. In the optimization experiment, the concentration of AMP was 13.5 ng/mL. 50 μL of AMP was added into 200 μL enhanced nanoflares and reacted in PBS buffer at 25 °C for 1h. F and F_0 represented the fluorescence intensity of enhanced nanoflares in the presence and absence of AMP, respectively.

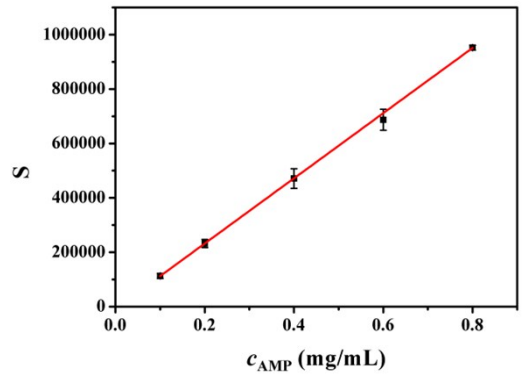

Fig. S6. Standard linear calibration curve (R^2 = 0.9998) for AMP detection with analytical standards for drug by HPLC.

Table S3. The results of AMP detection in drugs.

	Labelled amount (g/grain)	Measured amount (g/grain) Mean[a]	Measured as a percentage of labelled amount (%, n = 3)	RSD[b] (%, n=3)
HPLC	0.25	0.22	88	0.41
Our Method	0.25	0.21	84	0.76

a The mean of three determinations.

b RSD = Relative standard deviation

Table S4. The comparison of AMP detection between this work and other methods.

Methods	Linear range	LOD	Reference
HPLC	2-100 μg/mL (5.8-290 μM)	0.6 μg/mL (1.74 μM)	1
LC	0.4-200 μg/mL (1.15-290 μM)	-	2
Electrochemical	2.5-100 μM	1 μM	3
Electrochemical	1 fg/mL-2 ng/mL (29 fM-5.8 nM)	0.217 pg/mL (0.629 pM)	4
Electrochemical	5-5000 μM	1 μM	5
Colorimetric	1-60 nM	0.1 nM	6
Voltammetric	0.001-10 ng/mL (0.0029-29 nM)	0.3 pg/mL (0.87 pM)	7
Fluorescence	0.1-100 ng/mL (0.29-290 nM)	0.07 ng/mL (0.2 nM)	8
Fluorescence	0.001-10 ng/mL (0.0029-29 nM)	0.3 pg/mL (0.87 pM)	9
Fluorescence	0.5 -50 ng/mL (1.45-145 nM)	2 ng/mL (5.8 nM)	10
Fluorescence	1.8-20 ng/mL (5.2-58 nM)	0.65 ng/mL (1.9 nM)	This work

References

1. A. Aghaei, M. E. Jazi, T. Mlsna, M. A. Kamyabi, *J. Sep. Sci.*, 2019, **42**, 3002-3008.
2. F. A. Ibrahim and J. J. M. Nasr, *Anal. Methods*, 2014, **6**, 1523-1529.
3. F. Moura, F. G. de Almeida, B. R. Lopes, Q. B. Cass, *J. Sep. Sci.* , 2012, **35**, 2615-2620.
4. X. K. Liu, M. Y. Hu, M. H. Wang, Y. P. Song, N. Zhou, L. H. He and Z. H. Zhang, *Biosens. Bioelectron.*, 2019, **123**, 59-68.
5. Z. G. Yu and R. Y. Lai, *Talanta*, 2018, **176**, 619-624.
6. O. H. Shayesteh and R. Ghavami, *Mikrochim. Aacta*, 2019, **186**, 485-495.
7. J. Wang, K. Ma, H. S. Yin, Y. L. Zhou and S. Y. Ai, *Mikrochim. Acta*, 2017, **185**, 68-73.
8. Z. W. Luo, Y. M. Wang, X. Y. Lu, J. M. Chen, F. J. Wei, Z. J. Huang, C. Zhou and Y. X. Duan, *Anal. Chim. Acta*, 2017, **984**, 177-184.
9. Q. Yang, L. Y. Zhou, Y. X. Wu, K. Zhang, Y. T. Cao, Y. Zhou, D. Z. Wu, F. T. Hu and N. Gan, *Anal. Chim. Acta*, 2018, **1020**, 1-8.
10. K. M. Song, E. Jeong, W. Jeon, M. Cho and C. Ban, *Anal. Bioanal. Chem.*, 2012, **402**, 2153-2161.