Supporting Information

Fluorescence and colorimetric analysis of β-estradiol based on aptamer assembled spherical nucleic acids

Leyuan Chen [a,b,c], Aijiao Yuan [a,c], Dapeng Zhang [a,b,c,] Wenjing Xie [a,c] and Hanyong Peng [a,c*]

1. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China.
2. School of Environment, Hangzhou Institute for Academy Study, University of Chinese Academy of Sciences, Hangzhou 310024, China.
3. University of Chinese Academy of Sciences, Beijing 100049, China.

* Corresponding authors.

E-mail: hypeng@rcees.ac.cn

Telephone: +86 (010) 62849171

Table S1. List of oligonucleotide sequences

Name	Sequences（5’ to 3’）
A	SH-TTTTTTTTTTTTTTTAGTCGTCGAGAGAAA
B	SH-TTTTTTT**AAACTCTCGACGACT**AAA
Apt	ACGACTTAAGGTATGTGATCTTAGTTGTAGTTCA **AGTCGTCGAGAGTTT**-FAM
polyT	SH-TTTTTTTTTTTTTTT

Table S2. Comparison with other sensing methods for E2 detection

Methods	Linear range	R^2	LOD	Time	References
Fluorescence	0.35-35000 nM	0.985	0.35 nM	180 min	*1*
Fluorescence	5-75 nM	/	2.1 nM	10 min	*2*
Fluorescence	80-400 nM	0.98	37 nM	60 min	*3*
ELISA	1.5-50 nM	0.972	1.5 nM	/	*4*
Colorimetric	6.7 - 66700 nM	/	6.7 nM	50 min	*5*
Colorimetric	0.7-2000 nM	0.946	0.7 nM	70 min	*6*
Colorimetric	1.57-350 nM	0.995	1.57 nM	55 min	*7*
Fluorescence	0-250 nM	0.998	1 nM	60 min	This work
Colorimetric	10-500 nM	0.990	5 nM	50 min	This work

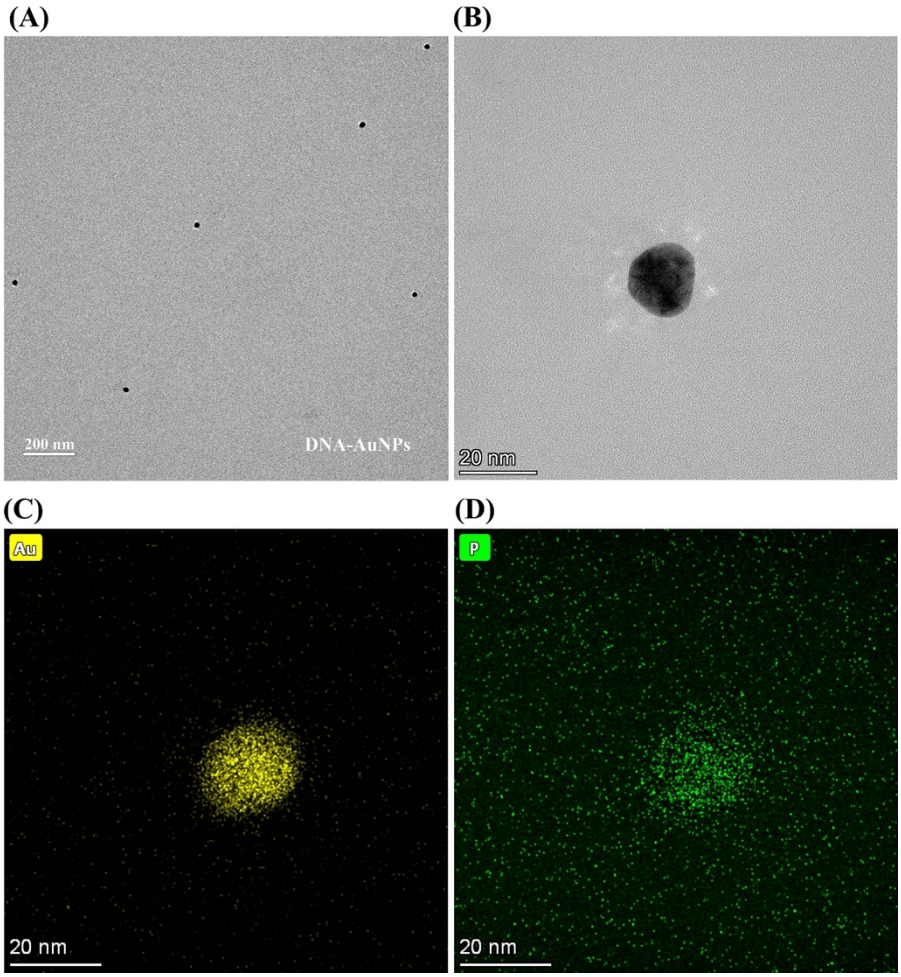

Figure S1. (A) TEM image of ssDNA-A-AuNPs at 200 nm scale. (B) TEM image of ssDNA-A-AuNPs at 20 nm scale. (C) EDX image of ssDNA-A-AuNPs for Au element. (D) EDX image of ssDNA-A-AuNPs for P element.

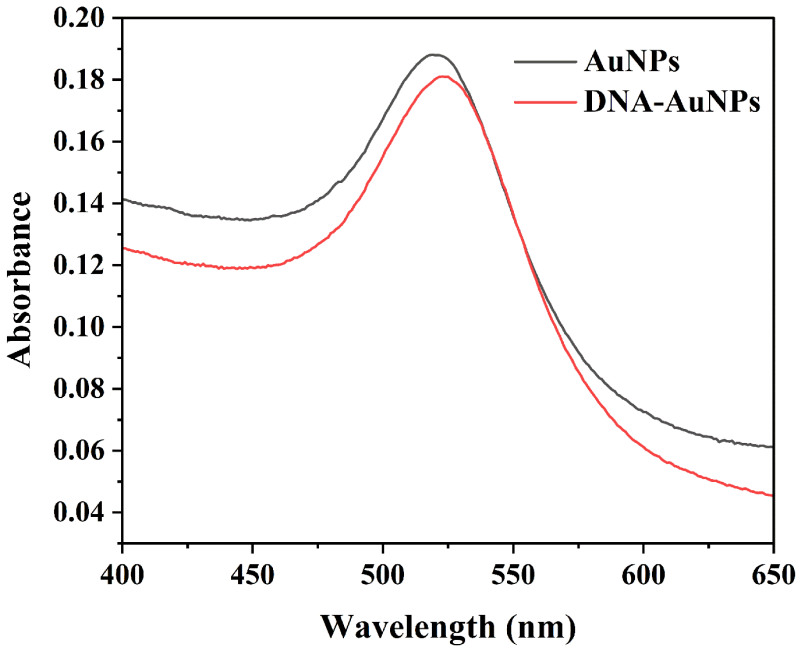

Figure S2. Absorption spectrum of 0.5 nM AuNPs and 0.5 nM dsDNA-AuNPs solutions. Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 100 mM NaCl.

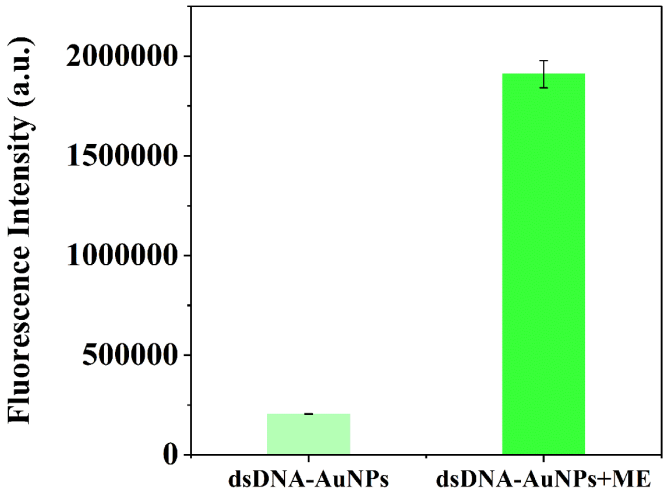

Figure S3. Fluorescence intensity of dsAuNPs-DNA solution with/without mercaptoethanol (ME). Concentration of dsDNA-AuNPs was 0.2 nM. The ME concentration in the detection solution was 5 mM. As mercaptoethanol (ME) has the capability to disrupt the disulfide bond that links DNA to AuNPs, the DNA molecules will detach from the AuNPs' surface upon ME addition. Consequently, the fluorescence quenching effect of FAM by AuNPs will be alleviated. This allows us to confirm the effective attachment of DNA onto the AuNPs' surface by assessing the fluorescence intensity of the AuNPs+DNA solution both with and without mercaptoethanol. The notable recovery in the fluorescence signal of the solution post-ME addition further indicates the successful DNA modification on the AuNPs' surface.

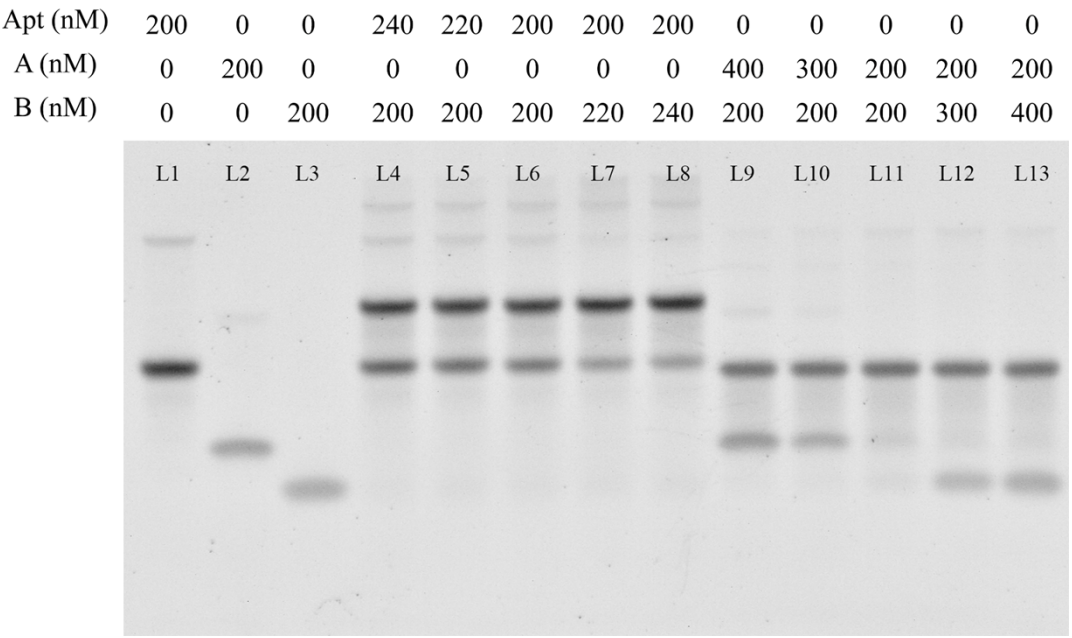

Figure S4. Electrophoresis results of Apt and ssDNA-B, ssDNA-A and ssDNA-B mixed in different concentration ratios. L1: 200 nM Apt; L2: 200 nM ssDNA-A; L3: 200 nM ssDNA-B; L4: 240 nM Apt + 200 nM ssDNA-B, Apt : ssDNA-B =1.2:1; L5: 220 nM Apt + 200 nM ssDNA-B, Apt : ssDNA-B =1.1:1; L6: 200 nM Apt + 200 nM ssDNA-B, Apt : ssDNA-B =1:1; L7: 200 nM Apt + 220 nM ssDNA-B, Apt : ssDNA-B =1:1.1; L8: 200 nM Apt + 240 nM ssDNA-B, Apt : ssDNA-B =1:1.2; L9: 400 nM ssDNA-A + 200 nM ssDNA-B, ssDNA-A : ssDNA-B=2:1; L10: 300 nM ssDNA-A + 200 nM ssDNA-B, ssDNA-A : ssDNA-B=1.5:1; L11: 200 nM ssDNA-A + 200 nM ssDNA-B, ssDNA-A : ssDNA-B =1:1; L12: 200 nM ssDNA-A + 300 nM ssDNA-B, ssDNA-A : ssDNA-B =1:1.5; L13: 200 nM ssDNA-A + 400 nM ssDNA-B, ssDNA-A : ssDNA-B =1:2.

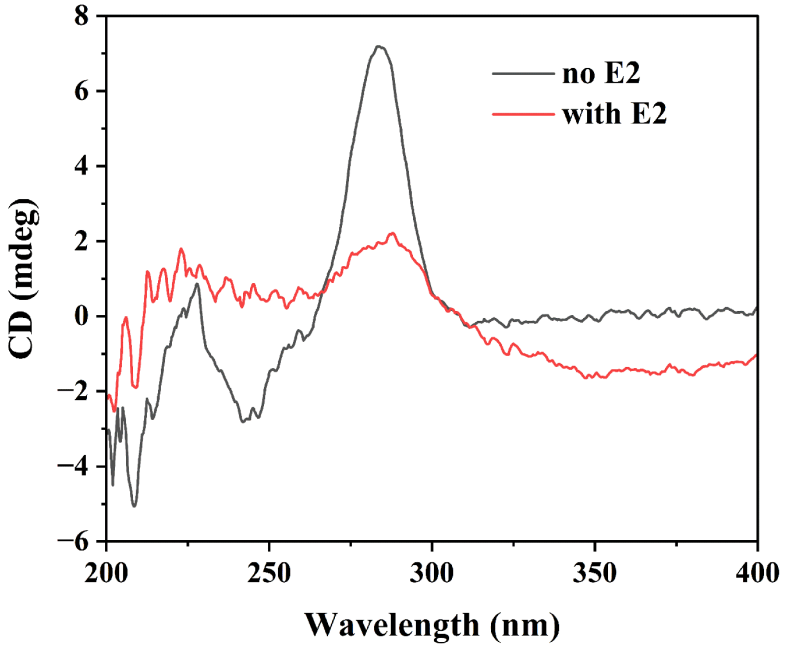

Figure S5. CD spectroscopy of dsDNA solutions before and after the addition of E2. Assay buffer was the sensing buffer at 25 ℃. Concentration of dsDNA was 4 μM. The E2 concentration in the detection solution was 1 mM.

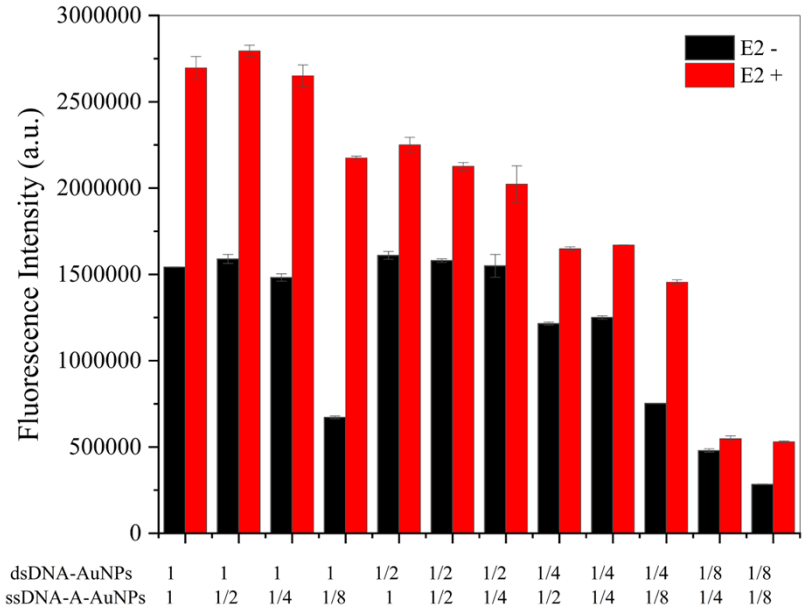

Figure S6. Optimization of loading capacity of ssDNA-A-AuNPs and dsDNA-AuNPs for E2 detection. The number at the bottom of the X-axis refers to dsDNA-AuNPs or ssDNA-A-AuNPs in the mixture with loading capacity of 1/n to the original.

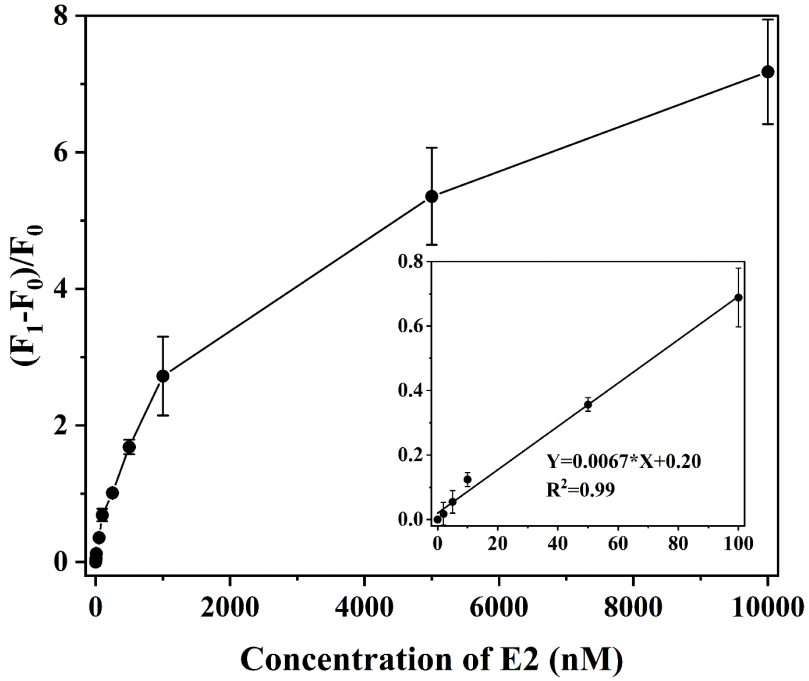

Figure S7. Relationship between the change of $(F_1-F_0)/F_0$ value and spiked E2 concentrations in 10-fold diluted tap water samples. The inset shows the $(F_1-F_0)/F_0$ value response to the concentration of E2 from 0 to 100 nM.

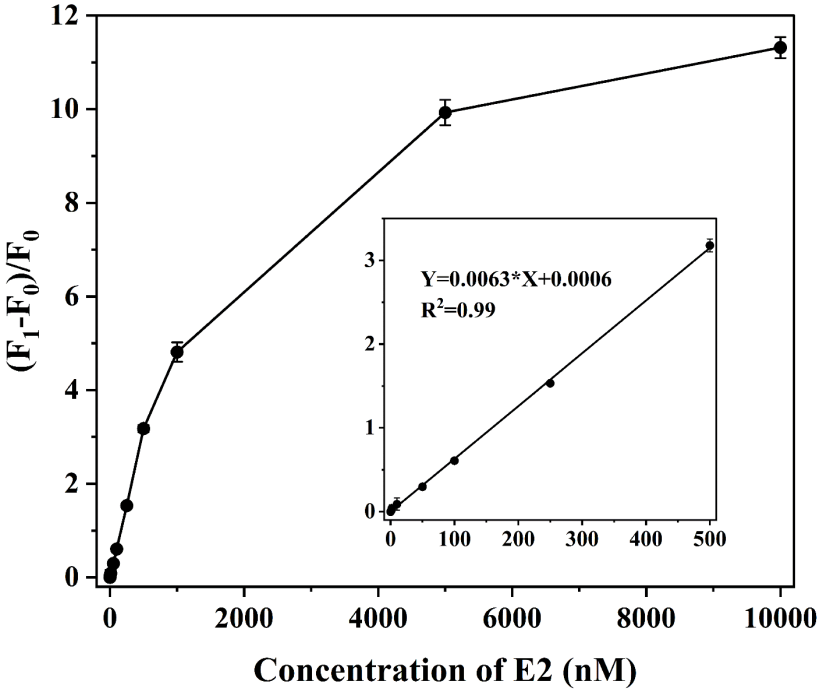

Figure S8. Relationship between the change of $(F_1-F_0)/F_0$ value and spiked E2 concentrations in 20-fold diluted FBS samples. The inset shows the $(F_1-F_0)/F_0$ value response to the concentration of E2 from 0 to 500 nM.

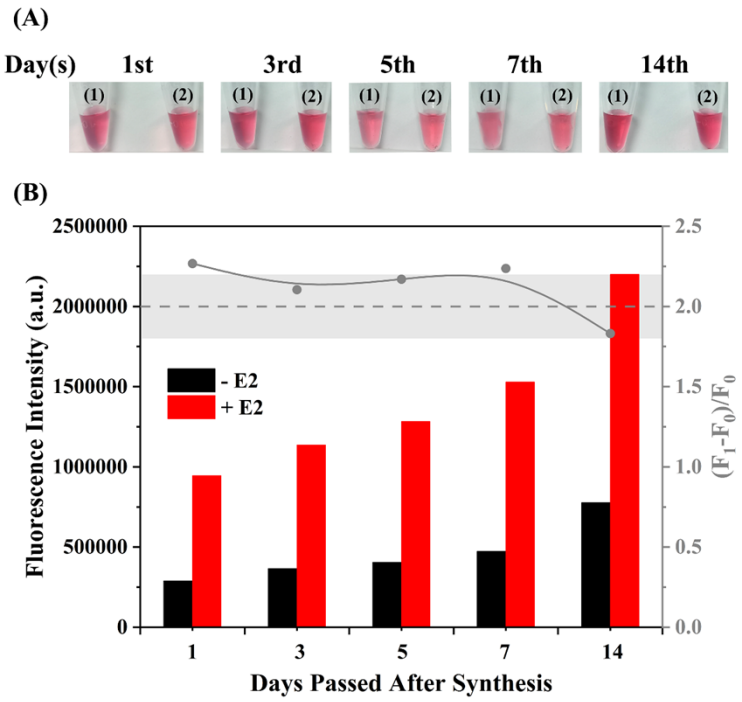

Figure S9. (A) Appearance changes of ssDNA-A-AuNPs and dsDNA-AuNPs within 14 days. Tube (1) refers to 2 nM dsDNA-AuNPs in preserving buffer; tube (2) refers to 2 nM ssDNA-A-AuNPs in preserving buffer. (B) Changes in E2 detection performance of biosensors within 14 days (add E2 concentration of 500 nM).

References

1 G. Zhang, T. Lia, J. Zhang and A. Chen, *Sens. Actuators, B*, 2018, **273**, 1648–1653.
2 N. Yildirim, F. Long, C. Gao, M. He, H. C. Shi and A. Z. Gu, *Environ. Sci. Technol.*, 2012, **46**, 3288−3294.
3 H. Huang, S. Shi, X. Gao, R. Gao, Y. Zhu, X. Wu, R. Zang and T. Yao, *Biosens. Bioelectron.*, 2016, **79**, 198–204.
4 Y. Huang, L. Zhang, Z. Li, S. C. B. Gopinath, Y. Chen and Y. Xiao, *Biotechnol. Appl. Bioc.*, 2020, **68**, 881–888.
5 C. C. Chang and C. Y. Yeh, *Anal. Sci.*, 2021, **37**, 479-483.
6 C. C. Chang, C. F. Li, Z. H. Yang, P. Y. Lin, H. C. Chang and C. W. Yang, *Sens. Actuators, B*, 2022, **364**, 131823.
7 D. Zhang, W. Zhang, J. Ye, S. Zhan, B. Xia, J. Lv, H Xu, G. Du and L. Wan, *Aust. J. Chem.*, 2016, **69**, 12-19.