Supporting Information

Detecting carcinoembryonic antigen based on aggregation-induced emission enhancement effect

Yanjun Lu,[a] Mengqi Wang,[a] Yiwen Yang,*[a] Qiukai Tang,[b] Yanbo Zeng,[a] Hailong Wang,[a] Lijie Zhang,[a] Chengrui Pan,[a] Changkun Hu,[a] Zhuowei Fu[a] and Lei Li*[a]

[a] Jiaxing Key Laboratory of Molecular Recognition and Sensing, College of Biological, Chemical Sciences and Engineering, Jiaxing University, Jiaxing 314001, Zhejiang, China.

[b] Clinical Laboratory, Zhejiang Sian International Hospital, Jiaxing 314031, Zhejiang, China

Corresponding author:

Yiwen Yang

E-mail: yangyiwen@mail.zjxu.edu.cn

Lei Li

E-mail: lei.li@mail.zjxu.edu.cn

1. Experimental section

1.1. Chemicals and Reagents.

Carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), and carbohydrate antigen 125 (CA125) were provided by Beijing Biosynthesis Biotechnology Co., Ltd. Human serum albumin (HSA) and bovine serum albumin (BSA) were purchased from Beijing J&K Science Co., Ltd. Immunoglobulin G (IgG) and vascular endothelial growth factor (VEGF) were obtained from Beijing Zhong Shan-Golden Bridge Biological Technology Co., Ltd. Protein tyrosine kinase-7 (PTK7) and immunoglobulin E (IgE) were purchased from Shanghai Hanxiang Biotechnology Co., Ltd. Prostate specific antigen (PSA) was obtained from Shanghai Linc-Bio Science Co., Ltd. CEA Apt (5′-ACTACAGCTTCAATT-3′) was provided by Sangon Biotech (Shanghai) Co., Ltd. Na_2HPO_4, NaH_2PO_4, [1,1'-biphenyl]-4,4'-diyldiboronic acid, and 4,4'-bis(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)-1,1'-biphenyl were provided by Shanghai Aladdin Biochemical Technology Co., Ltd. Methanol was provided by Sinopharm Chemical Reagent Co., Ltd. All chemicals used are of analytical grade and can be used without further purification. Ultrapure water was used in each experiment.

1.2. Instrumentation.

The fluorescence spectrum was recorded by an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence lifetime was obtained on an FLS980 transient fluorescence spectrometer (Edinburgh, UK).

1.3 Preparation of the CEA-Apt/A complex

First, 40 μL of 50 μM Compound A solution, 20 μL of 10 μM (or 0.02, 0.1, 0.2, 1, 2, 5, and 20 μM) CEA-Apt solution, and 340 μL of 0.1 M PBS (pH = 7.4) were mixed evenly. After incubating the mixed solution at 37 ℃ for 1–10 min, the fluorescence intensity of the mixture was measured.

1.4 Detection of CEA

First, 40 μL of 50 μM compound A solution and 20 μL of 10 μM CEA-Apt solution were mixed evenly. After incubation at 37 ℃ for 5 min, the fluorescence intensity of the mixture reached its maximum value. Then, 40 μL of different concentrations (0.03, 1, 10, 20, 30, 50, 70, and 100 ng/mL) of CEA and 300 μL of 0.1 M PBS (pH = 7.4) were added to the abovementioned mixture and mixed evenly. After incubation at 37 ℃ for 50 min, the fluorescence intensity of the mixed solution was tested.

1.5 Specificity and anti-interference of the aptasensor

AFP, IgG, VECF, HSA, BSA, CA125, IgE, PSA, and PTK7 were selected to evaluate the selectivity and anti-interference of aptasensors.

1.6 Practicability of the aptasensor

To evaluate the practicality of the aptasensor in complex biological matrices, the level of CEA in serum samples from patients with cancer was measured. First, the patient's serum was diluted 10 times with 0.1 M PBS (pH = 7.4) and then detected in accordance with the method presented in Section 1.4. The human serum samples were obtained from Zhejiang Sian International Hospital.

2. Supplemental Figures

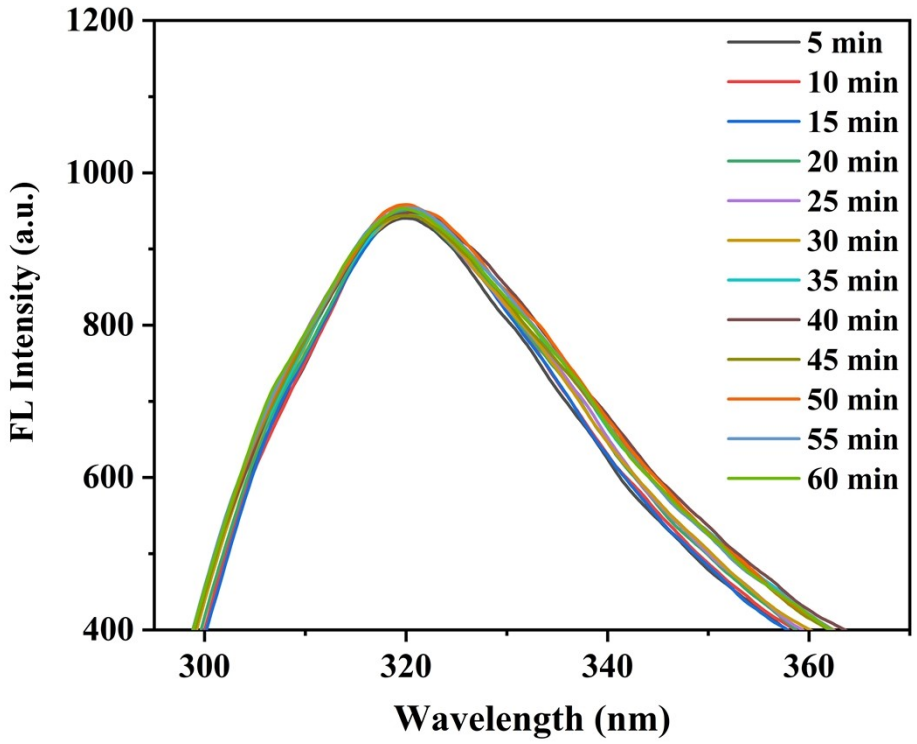

Fig. S1 Fluorescence emission spectra of 5 μM Compound A acquired over 60 min after excitation at 270 nm (λ_{em} = 320 nm).

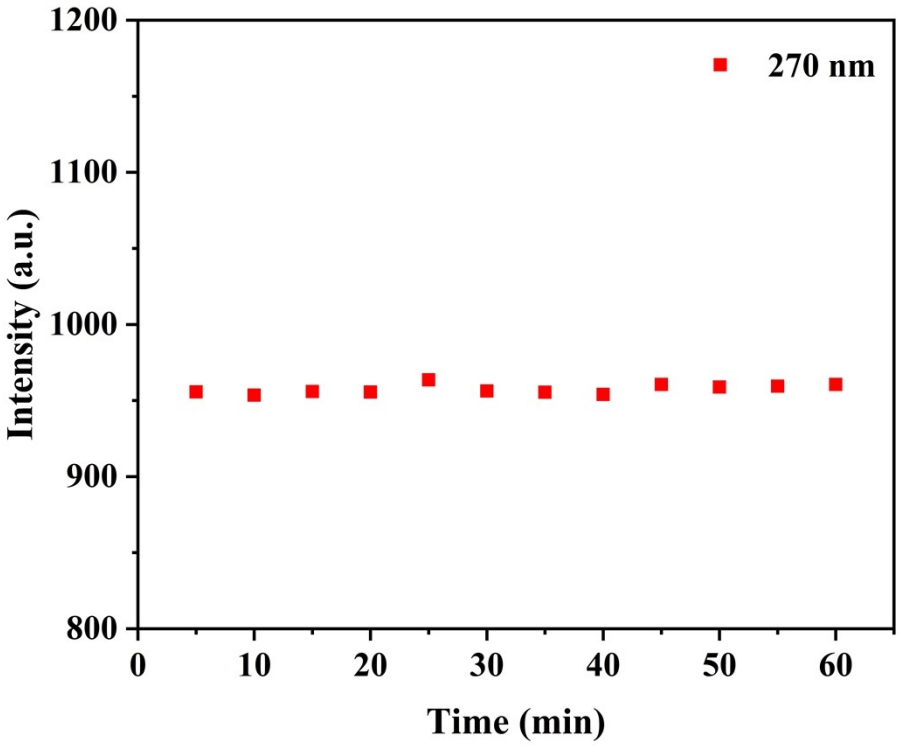

Fig. S2 Fluorescence intensity of 5 μM Compound A acquired over 60 min after excitation at 270 nm (λ_{em} = 320 nm).

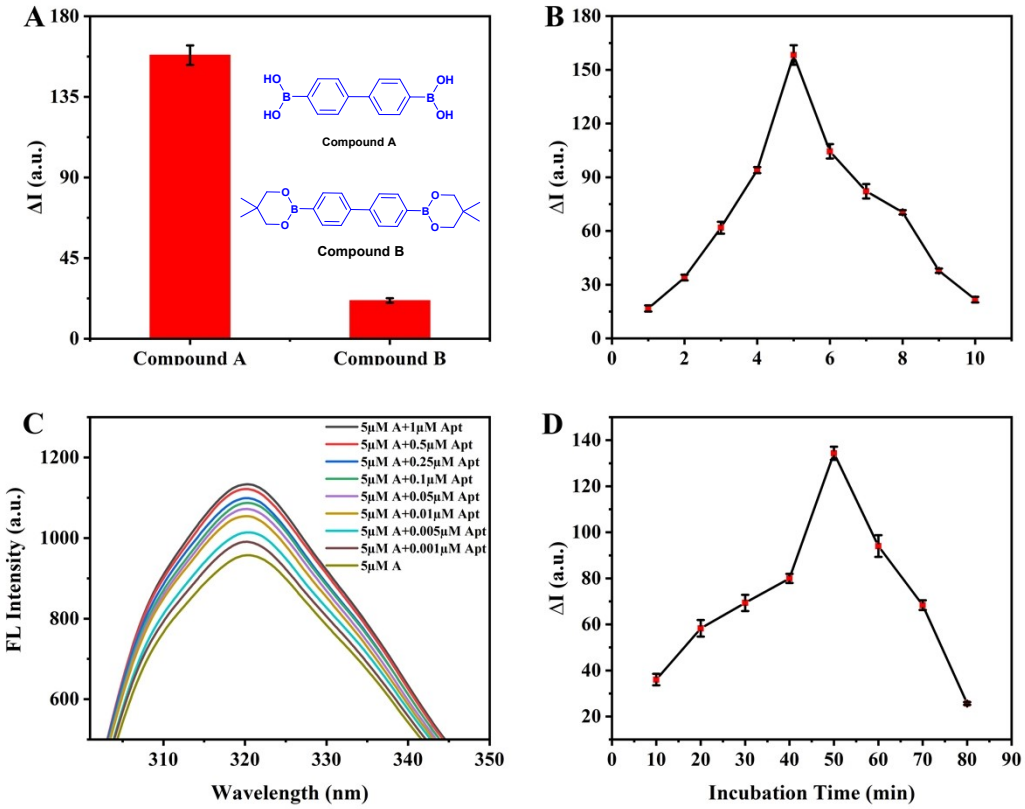

Fig. S3 (A) Increase in the fluorescence intensity of Compound A (or B; 5 μM) mixed with CEA-Apt (0.5 μM; Compound A: λ_{ex} = 270 nm and λ_{em} = 320 nm; Compound B: λ_{ex} = 270 nm and λ_{em} = 320 nm). (B) Effect of the incubation time of CEA-Apt and Compound A on the fluorescence intensity of Compound A ($\Delta I = I - I_0$, I_0, and I were the fluorescence intensities without CEA-Apt and with CEA-Apt). (C) Effect of CEA-Apt concentration on the fluorescence intensity of Compound A (λ_{ex} = 270 nm, λ_{em} = 320 nm). (D) Effect of CEA incubation time on the fluorescence intensity of Compound A ($\Delta I = I_0 - I$, I_0, and I were the fluorescence intensities without CEA and with CEA).

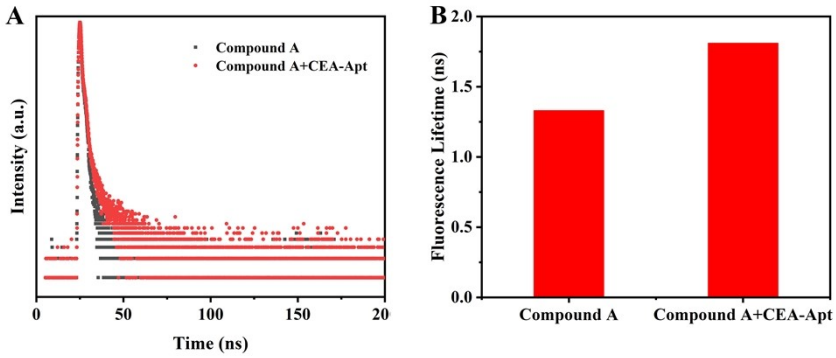

Fig. S4 (A) Fluorescence lifetime decay curve of Compound A in 5 μM Compound A solution or a mixture of 5 μM Compound A and 0.5 μM CEA-Apt; (B) Fluorescence lifetime of Compound A in 5 μM Compound A solution or a mixture of 5 μM Compound A and 0.5 μM CEA-Apt.

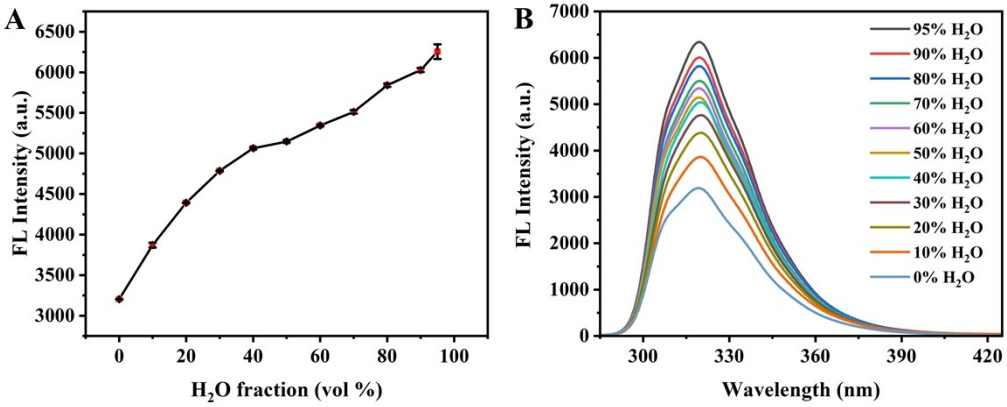

Fig. S5 (A) Fluorescence intensity of Compound A in MeOH/H_2O-mixed solutions with different volume fractions of H_2O. (B) Fluorescence spectra of Compound A in MeOH/H_2O-mixed solutions with different volume fractions of H_2O.

Table S1 Comparison between the proposed method and other CEA detection methods

No.	Method	LOD (ng mL^{-1})	Linear range (ng mL^{-1})	References
1	Bimetallic organic framework Cu/UiO-66 fluorescence method	0.01	0.01–0.3	[1]
2	Photoelectrochemical immunoassay	0.0035	0.01–40	[2]
3	Electrochemiluminescence biosensor	2.51	5–300	[3]
4	Lateral flow immunoassay	0.023	-	[4]
5	Abiotic fluorescent probe	0.2	0–200	[5]
6	DNA nanostructure-assisted detection	10.8	18–1800	[6]
7	Electrochemical sensor	0.08	0.1–100000	[7]
8	Fluorescence immunoassay	0.05	0.1–50	[8]
9	Lateral flow assay	0.0144	0.01–0.1	[9]
10	Electrochemiluminescence immunosensor	0.0029	0.005–500	[10]
11	Fluorescence aptasensors	0.001	0.003–10	This work

References

1. Y. Wu, X. Chen, X. Luo, M. Yang, C. Hou and D. Huo, *Anal. Chim. Acta*, 2021, **1183**, 339000.
2. X. Wang, H. Wang, X. Wan, M. Li and D. Tang, *Anal. Chim. Acta*, 2023, **1279**, 341826.
3. M. Mohammadniaei, M. Zhang, X. Qin, W. Wang, L. Pia, H. Gürbüz, S. H. Helalat, M. Naseri and Y. Sun, *Talanta*, 2024, **266**, 125087.
4. Y. Wang, J. Shen, R. Song, Q. Xu, X. Hu and Y. Shu, *Talanta*, 2024, **266**, 125017.
5. J.-S. Lee, I.-h. Song, S. D. Warkad, G. S. Yeom and S. B. Nimse, *Bioorg. Chem.*, 2022, **119**, 105490.
6. R. Tian, T. Weng, S. Chen, J. Wu, B. Yin, W. Ma, L. Liang, W. Xie, Y. Wang, X. Zeng, Y. Yin and D. Wang, *Bioelectrochemistry*, 2023, **149**, 108284.
7. L. P. T. Carneiro, N. S. Ferreira, A. P. M. Tavares, A. Pinto, A. Mendes and M. G. F. Sales, *Biosens. Bioelectron.*, 2021, **175**, 112877.
8. X. Liu, N. Wang, Y. Hou, H. Dong, W. Liang, X. Li and Y. Yuan, *Chem. Commun.*, 2024, **60**, 3978-3981.
9. J. Fox, D. V. B. Batchelor, H. Roberts, S. C. T. Moorcroft, E. M. A. Valleley, P. L. Coletta and S. D. Evans, *ACS Appl. Nano Mater.*, 2023, **6**, 17769-17777.

10. Y. Tang, B. Zhang, Y. Wang, F. Zhao and B. Zeng, *ACS Appl. Nano Mater.*, 2021, **4**, 7264-7271.