Supplementary Material

for the manuscript:

Electrochemiluminescence aptasensor for amplified detection of exosomes from breast tumor cells (MCF-7 cells) based on G-quadruplex/hemin DNAzyme

Bin Qiao, [a] Qunqun Guo, [a] Juqian Jiang, [a] Yunlong Qi, [a] Hui Zhang [a,*], Bangshun He[b,*], Chenxin Cai [a] and Jian Shen [a,*]

[a] Jiangsu Key Laboratory of New Power Batteries, Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Laboratory of Biomedical Materials, National and Local Joint Engineering Research Center of Biomedical Functional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, P. R. China

[b] General Clinical Research Center, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, P. R. China.

* Corresponding authors.

Email address: zhangh@njnu.edu.cn; hebangshun@163.com; jshen@njnu.edu.cn;

S1 Experimental details

S1.1 Materials and Chemicals

Hydrogen peroxide (H_2O_2; 30%) was obtained from Sinopharm Chemistry Reagent Co., Ltd. (Shanghai, China). 3-Mercaptopropionic acid (MPA; 99%), bovine serum albumin (BSA), sodium sulfide ($Na_2S·9H_2O$; 98%), cadmium nitrate tetrahydrate ($Cd(NO_3)_2·4H_2O$), europium(III) nitrate hexahydrate ($Eu(NO_3)_3·6H_2O$) and hemin were purchased from Aladdin, N-(3-dimethylaminopropyl)-N'- ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade. Ultrapure water from a Millipore water system (resistance 18.2MΩ/cm) was used to prepare aqueous solutions throughout the experiments.

The modified specific oligonucleotide (Sequences:CD63 aptamer:5'-CAC CCC ACC TCG CTC CCG TGA CAC TAA TGC TAT TTT TTT TTT- $(CH_2)_7$-NH_2-3'; S1:5'-GGG TTG GGC GGG ATG GGT TTT TTC ACC CCA CCT CGC TCC CGT GAC ACT AAT GCT A-3') were synthesized by Sangon Bioengineering Ltd. (Shanghai, China).

Transmission electron microscopy (TEM) images were obtained using a JEOL-2010 transmission electron microscope with an accelerating voltage of 120 kV. The UV-vis absorption spectra were recorded with a

Cary 5000 UV-vis-NIR spectrophotometer (Varian, USA). EIS analyses were carried out with an Autolab PGSTAT-30 potentiostat/galvanostat (Eco Chemie BV, Utrecht, The Netherlands) in 0.1 M KCl containing 5.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ using a conventional three-electrode system. The electrochemical and ECL emission measurements were performed on a MPI-A multifunctional analytical system (Xi'An Remax Electronic Science & Technology Co. Ltd, Xi'An, China) using a three-electrode system with a platinum wire as the counter electrode, a modified GCE electrode as the working electrode and a saturated calomel (SCE) as the reference electrode.

S1.2 Synthesis of MPA-CdS:Eu NCs

112.5 μL of 0.08 M $Eu(NO_3)_3$ homogeneous solution was added to 30 mL of $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) aqueous solution with continuous stirring. Then 80 μL MPA was mixed with above solution; then NaOH was added to adjust the pH to the desired value of 10. Finally, the freshly prepared $Na_2S \cdot 9H_2O$ (0.7205 g) solution was dropped into the mixture, and the obtained solution turned orange-yellow. The solution was allow to react at 70 °C for 3 h with continuous stirring. The final reaction solution was centrifuged and then washed thoroughly with absolute ethanol three times, followed by washing with ultrapure water. Then the resulting precipitate was ultrasonically dispersed in water to form the

yellow MPA-CdS:Eu NC solution. The final solution could be stored for more than 1 month in a refrigerator at 4 °C.

S1.3 Cell culture

MCF-7 cells was cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% penicillin/streptomycin, and incubated at 37 °C in an atmosphere containing 5% CO_2.

S1.4 Quantification of exosomes by Nanoparticle Tracking Analysis (NTA)

First, the obtained exosome pellets were diluted with sterile PBS and analyzed by Nanoparticle Tracking Analysis (NTA). The exosome concentrations obtained from MCF-7 were 1.7×10^5 particle/μL. Theobtained exosome pellets were stored at -20 °C for further use.

S1.5 Transmission Electron Microscopy (TEM)

First, 10 μL of freshly prepared exosomes was placed onto a carbon-coated copper grid for 1min and then the remaining solution was absorbed by filter paper. Next, 10 μL of uranyl acetate solution was also placed onto the grids for another 1min. Then the remaining liquid was removed using filter paper and dried at room temperature [1]. Finally, the

prepared sample was observed under a transmission electron microscope[2].

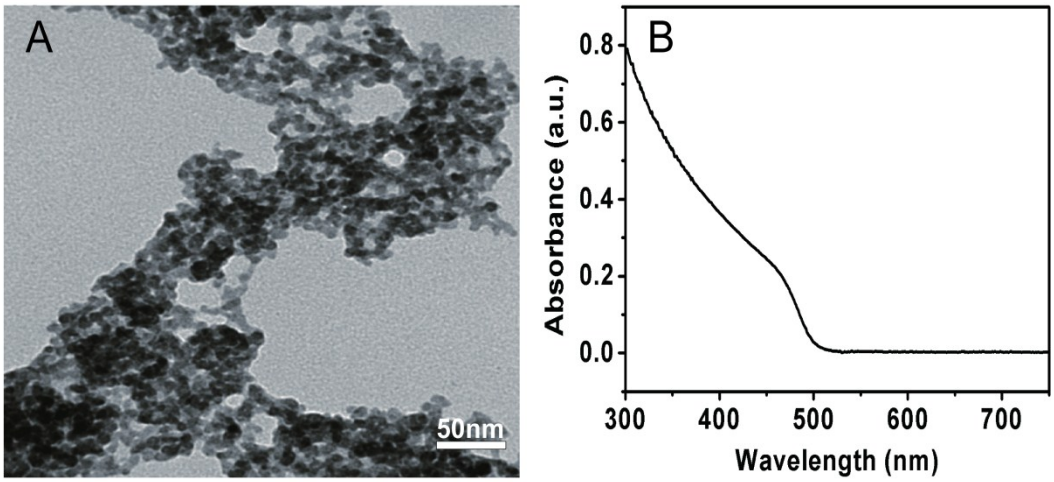

Fig. S1. Characterization of the MPA-CdS:Eu NCs. (A)TEM image and (B) UV-vis absorption spectra of the as-prepared CdS:Eu NCs.

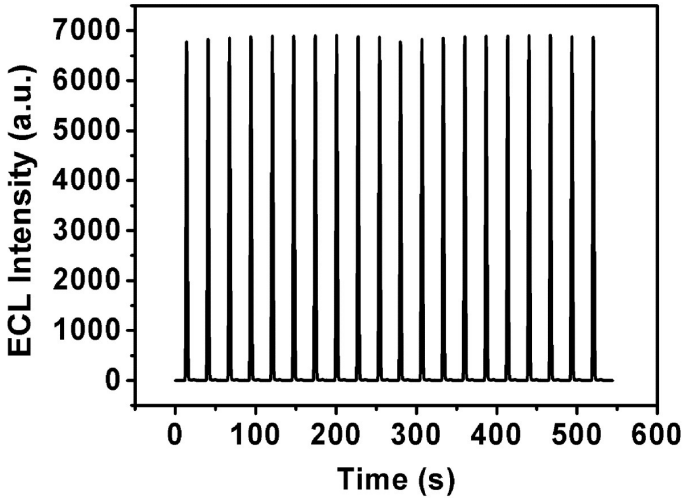

Fig. S2. ECL-time curve of MPA-CdS:Eu NCs in 0.10 M K^+, pH 8.5 PBS containing 30 mM H_2O_2 under continuous cyclic potential scan for 20 cycles from 0 to 1.35 V (vs.SCE) at a scan rate of 0.1 V/s.

References

[1] H. Shao, J. Chung, L. Balaj, A. Charest, D.D. Bigner, B.S. Carter, F.H. Hochberg, X.O. Breakefield, R. Weissleder, H. Lee, Nat. Med. 2012, **12**, 1835-1840.

[2] H. Dong, H. Chen, J. Jiang, H. Zhang, C. Cai, Q. M. Shen, Anal. Chem. 2018, **7**, 4507-7513.