

Supporting Information

Ultrasensitive Detection of Tumor-Derived Small Extracellular Vesicles Based on Nonlinear Hybridization Chain Reaction Fluorescence Signal Amplification and Immunomagnetic Separation

Qianqian Kong,^a Shasha Cheng,^a Xinyu Hu,^a Jia You,^a Cuiling Zhang^{*a} and Yuezhong Xian^{*a}

General experimental section

Figure S1. Absorption intensities at 450 nm for bare (control) and MBs-CD63.

Figure S2. Fluorescence spectra for sEVs detection *via* nHCR with or without free CD63 protein.

Figure S3. Fluorescence spectra corresponding to lanes 6 and 7 in Figure 3 (B).

Table S1. Sequences of oligonucleotides used in this work.

Table S2. Comparison of the present method with other approaches for sEVs detection.

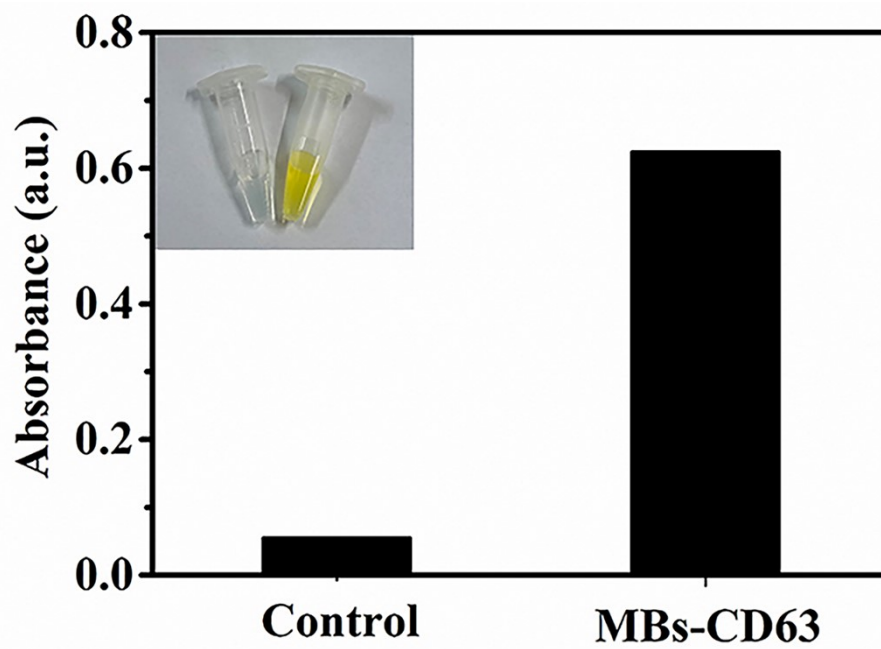


Figure S1. Absorption intensities at 450 nm for bare (control) and MBs-CD63. Inset is the corresponding digital photo.

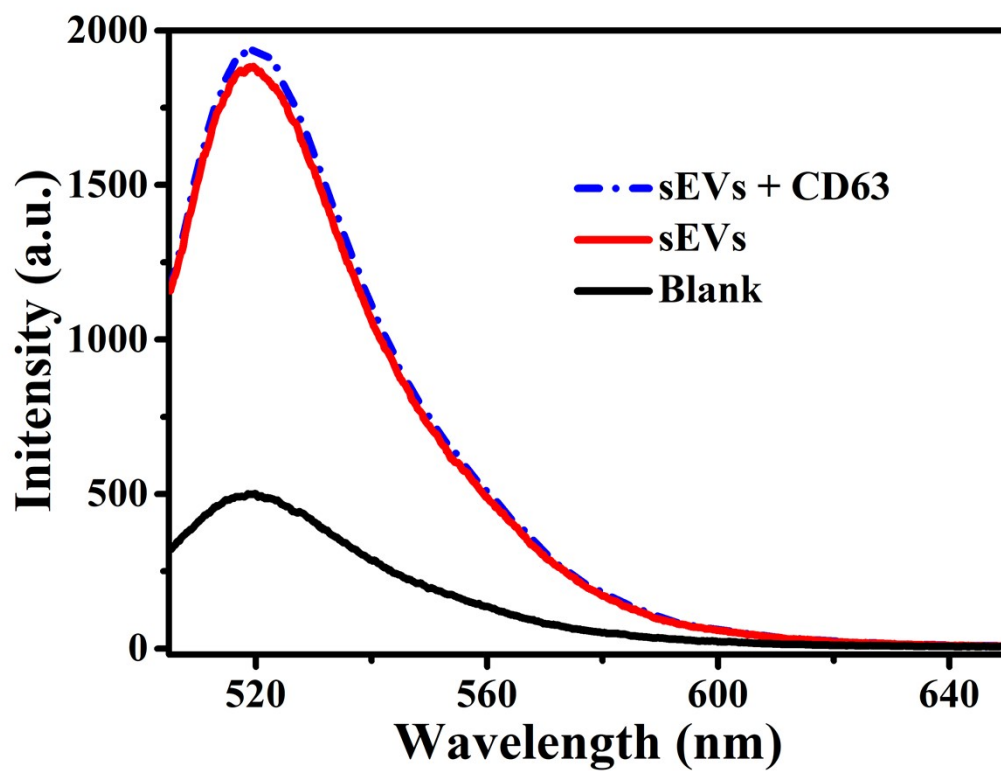


Figure S2. Fluorescence spectra for sEVs detection *via* nHCR with or without free CD63 protein.

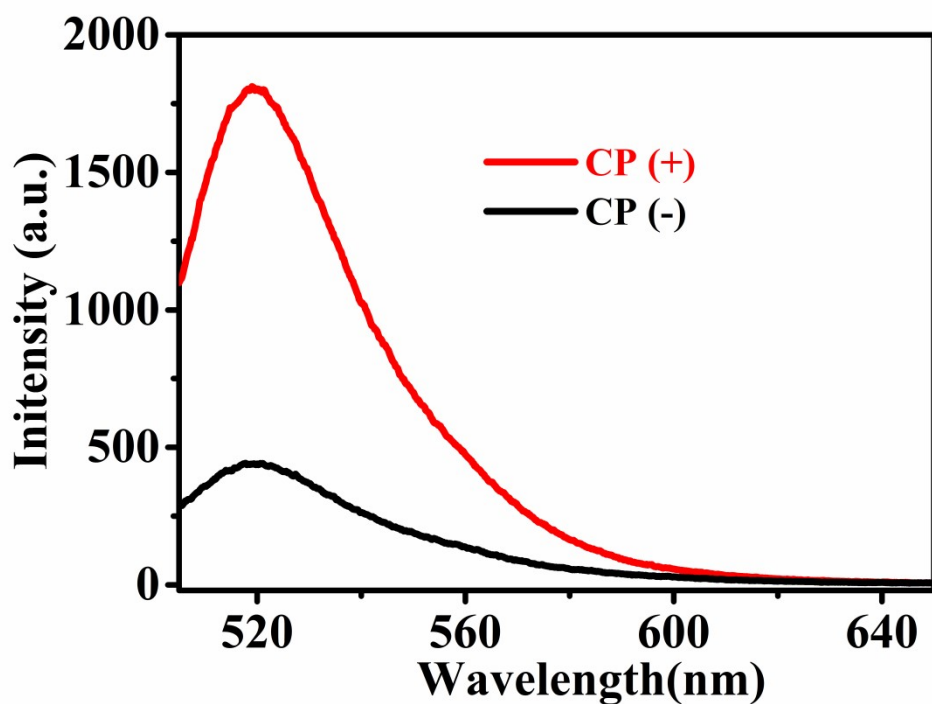


Figure S3. Fluorescence spectra corresponding to lanes 6 and 7 in **Figure 3 (B)**.

Table S1. Summary of DNA sequence information used in this work

Name (abbreviation)	Sequences (5' to 3')	Modification
H1	TTTTTTTTTTTTTTAACCGAATCCTAGACTCAAAGTAGTCTAG GATTC	5'Biotin, 3'FAM
H2	TTTTTTTTTTTTTTAGTCTAGGATTCGGTTAAGAATCCTAGACTA CTTG	5'Biotin, 3'FAM
CP	AGTCTAGGATTCGGTTAA	3'cholesterol
CCP	GAATCCTAGACT	3'FAM

Table S2. Comparison of the present method with other approaches for sEVs detection.

Detection Method	Sensing Strategy	LOD (particles/ μ L)	Linear range (particles/ μ L)	Ref.
Fluorescence	GO nanoprobe / enzyme-mediated signal amplification	1.6×10^2	$1.6 \times 10^2 - 1.6 \times 10^5$	1
Fluorescence	Dual signal amplification platform based on rolling circle amplification	1.0×10^2	$1.0 \times 10^3 - 1.0 \times 10^5$	2
Fluorescence	Bivalent cholesterol anchored enzyme-free DNA circuits signal amplification	1.29×10^3	$5.5 \times 10^3 - 1.1 \times 10^7$	3
Fluorescence	TdT-catalyzed polymerization based on steric hindrance-controlled signal amplification	4.8×10^2	$1.66 \times 10^3 - 1.66 \times 10^6$	4
Fluorescence	Bicolor exosomal phenotyping based on multivalence-actuated DNA nanomachines	33	$4.5 \times 10 - 4.5 \times 10^5$	5
Electrochemistry	Aptamer-based exonuclease III (Exo III)-assisted recycling amplification	12	$3.4 \times 10^1 - 3.4 \times 10^5$	6
Electrochemistry	Electrochemical aptasensor based on click chemistry and the DNA HCR for signal amplification	96	$1.12 \times 10^2 - 1.12 \times 10^8$	7
Colorimetry	Enzyme-catalyzed metallization of Au nanorods and hybridization chain reaction	1.6×10^2	$1.4 \times 10^3 - 9.0 \times 10^6$	8
Colorimetry	HRP-catalyzed H_2O_2 -mediated oxidation of TMB	2.2×10^3	$1.8 \times 10^4 - 7.9 \times 10^5$	9
Raman scattering	A novel Raman probe based on self-assembly of gold nanoparticles in triangular pyramid DNA	1.1×10^2	$3.125 \times 10^4 - 1.0 \times 10^6$	10
Electrochemiluminescence	Aptamer-binding DNA walking machine for signal amplification	60	$2.0 \times 10^2 - 7.5 \times 10^4$	11
Fluorescence	Nonlinear HCR fluorescence signal amplification based on immunomagnetic separation	80	$2.0 \times 10^2 - 8.0 \times 10^6$	This work

References

1. D. Jin, F. Yang, Y. Zhang, L. Liu, Y. Zhou, F. Wang and G. J. Zhang, *Anal. Chem.* 2018, **90**, 14402–14411.
2. L. Huang, D. B. Wang, N. Singh, F. Yang, L. Hai, N. Gu and X. E. Zhang, *Nanoscale*, 2018,**10**, 20289-20295.
3. X. Wang, H. Shang, C. Ma and L. Chen, *Anal. Chem.* 2021, **93**, 8493-8500.
4. Y. Pan, L. Wang, Y. Deng, M. Wang, Y. Peng, J. Yang and G. Li, *Chem. Commun.* 2020, **56**, 13768–13771.
5. D. Jin, X. X. Peng, Y. Qin, P. Wu, H. Lu, L. Wang, J. Huang, Y. Li, Y. Zhang, G. Zhang and F. Yang, *Anal. Chem.* 2020, **92**, 9877-9886.
6. X. Yin, T. Hou, B. Huang, L. Yang and F. Li, *Chem. Commun.* 2019, **55**, 13705-13708.
7. Y. An, T. Y. Jin, Y. Y. Zhu, F. Zhang and P. G. He, *Biosens. Bioelectron.* 2019, **142**,111503.
8. Y. Z. Zhang, D. N. Wang, S. Yue, Y. B. Lu, C. G. Yang, J. Fang and Z. R. Xu, *ACS Sens.* 2019, **4** (**12**), 3210-3218.
9. F. He, H. Liu, X. G. Guo, B. C. Yin and B. C. Ye, *Anal. Chem.* 2017, **89** (**23**), 12968-12975.
10. X. Zhang, C. Liu, Y. Pei, W. Song and S. Zhang, *ACS Appl. Mater. Interfaces.* 2019, **11**, 28671–28680.
11. Q. M. Feng, P. Ma, Q. H. Cao, Y. H. Guo and J. J. Xu, *Chem. Commun.* 2020, **56**, 269–272.