Supplementary Information

A novel surface-enhanced Raman scattering probe based on Au nanoboxes for dynamic monitoring caspase-3 during cervical cancer cells apoptosis

Yue Sun,[af] Youwei Wang,[b] Wenbo Lu,[c] Chang Liu,[e] Shengjie Ge,[af] Xinyu Zhou,[af]

Caili Bi[f]* and Xiaowei Cao[adf]*

[a]Institute of Translational Medicine, Medical College, Yangzhou University, Yangzhou, 225001, P. R. China.

[b]Department of neurosurgery, Affiliated Hospital of Yangzhou University, Yangzhou, 225001, P. R. China.

[c]Shanxi Normal University, College of Chemistry and Material Science, Linfen, 041004, P. R. China.

[d]Jiangsu Key Laboratory of Zoonosis, Yangzhou University, Yangzhou, 225009, P. R. China.

[e]School of Grain Science and Technology, Jiangsu University of Science and Technology, Zhenjiang, 212000, P. R. China.

[f]Jiangsu Key Laboratory of Experimental & Translational Non-coding RNA Research, Medical College, Yangzhou University, Yangzhou, 225001, P. R. China.

*Corresponding authors

E-mail: cxw19861121@163.com, bcl2018@yzu.edu.cn

1. Results and discussion

1.1 Characterization of Au nanoboxes

Fig. S1A showed the energy dispersive X-ray spectroscopy (EDS) elemental mapping of the Au nanoboxes. The weight ratio of the gold and silver elements was 83.38% and 16.62%, respectively. And the ratio of gold to silver in nanoboxes was around 5. It can be seen that the products were basically Au nanoboxes and small number of products were Au-Ag alloy. Fig. S1B recorded the SERS spectra of the NBA and NBA-labeled Au nanoboxes. The SERS enhancement factor (EF) for Au nanoboxes was calculated by using $EF=(I_{SERS}/C_{SERS})/(I_{RS}/C_{RS})$,[1] where I_{SERS} denoted the SERS intensity of the Au nanoboxes colloidal dispersion at a certain concentration of the analyte, C_{SERS}; I_{RS} denoted the Raman intensity at analyte concentration of C_{RS} under non-SERS conditions. NBA was selected as Raman report molecule, which characteristic peak located at 592 cm^{-1}. When $C_{SERS}=1\times10^{-6}$ M and $C_{RS}=10^{-2}$ M, the intensities at 592 cm^{-1} were measured as shown in Fig. S1 and the SERS EF of Au nanoboxes was obtained as 1.52×10^{5}. The prominent SERS enhancement effect may result from the excellent surface plasmon resonance effect of Au nanoboxes with hollow interior and porous walls, which depended on the coupling between their interior and exterior surface fields.[2]

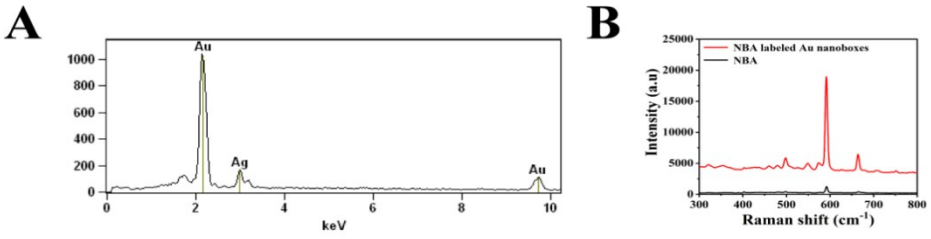

Fig. S1 (A) The EDS spectrum of the Au nanoboxes; (B) SERS spectra of the NBA and NBA-labeled Au nanoboxes.

1.2 Characterization of Au nanoboxes-NBA-peptide probes

The size changes during probes preparation could be facilely and directly characterized with the dynamic light scattering (DLS) technique (Fig. S2). Hydrodynamic diameters of nanoparticles and probes were investigated using Malvern™ Zetasizer Nano ZS (United Kingdom) equipped 4.0 mW red laser and a detection angle of 173°. The DLS results depicting the average hydrodynamic diameters of Au nanoboxes and Au nanoboxes-NBA-peptide were observed to be around 80 nm and 100 nm, respectively. The diameter of Au nanoboxes-NBA-peptide increased compared with bare Au nanoboxes due to the conjugation of NBA and peptide onto Au nanoboxes. In conclusion, all these results confirm that NBA and peptide were successfully modified on the surface of Au nanoboxes.

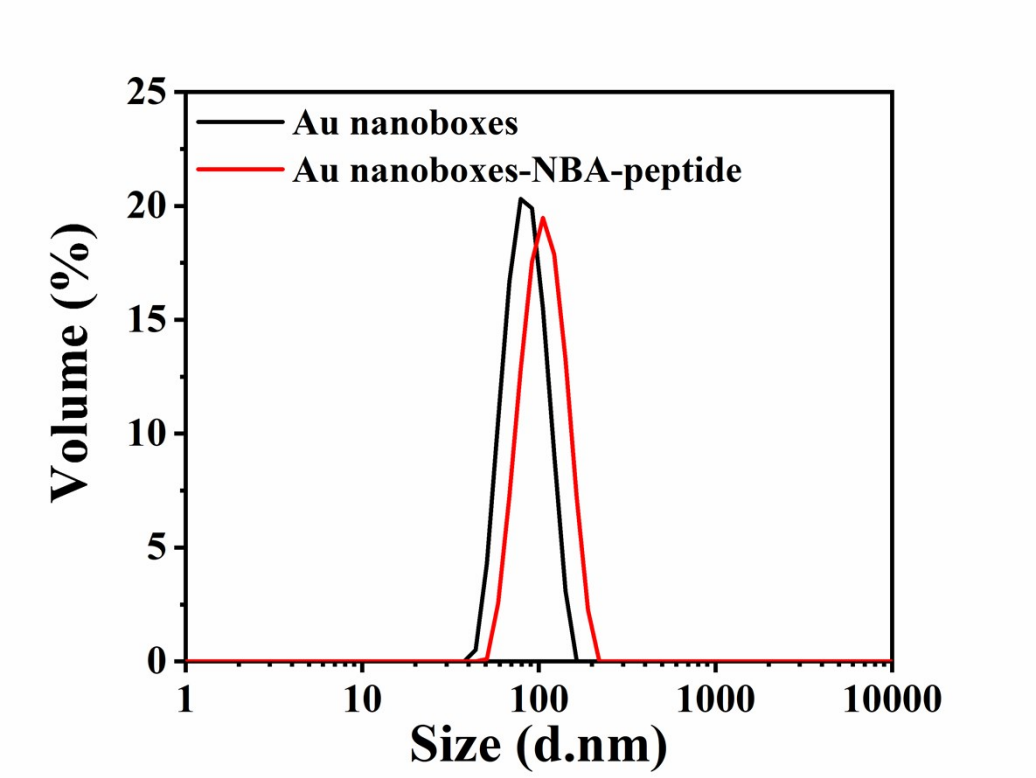

Fig. S2 Dynamic light scattering of Au nanoboxes and Au nanoboxes-NBA-peptide.

1.3 Optimization of the detection conditions

Because the signal intensity was strongly related to the number of NBA adsorbed on the surface, NBA concentration was optimized in the presence of 10 fM target caspase-3. As to NBA, when its concentration increased, the SERS intensity increased gradually and then trended to a constant value, due to the adsorption amount of NBA which directly affected the SERS signal gradually increased and tended to saturate. As shown in Fig. S3, the maximum SERS intensity can be achieved when 1 mM of NBA was used, which exhibited the optimized detection conditions.

The essence of caspase-3 is protein, which is prone to denaturation when exposed to an unfavorable acidic or alkaline environment. As shown in Fig. S4, the influence of pH values ranging from 6.0 to 8.0 on the SERS signal intensities produced by 10 fM caspase-3 was observed. And the SERS intensity reached a maximum at pH 7.4. Therefore, we selected pH 7.4 as the optimum pH value.

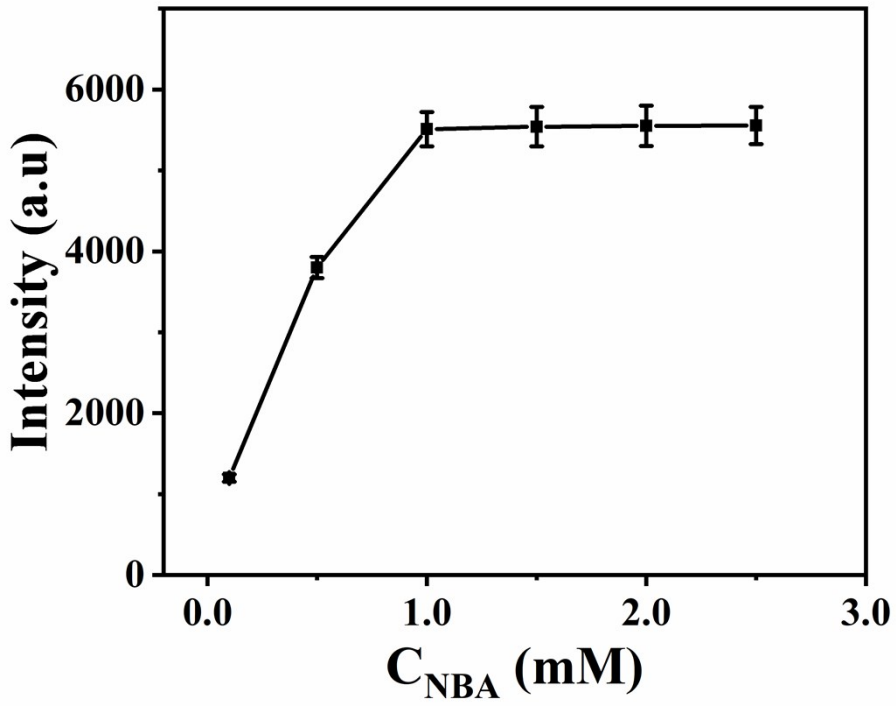

Fig. S3 Effect of NBA concentration on the SERS intensities.

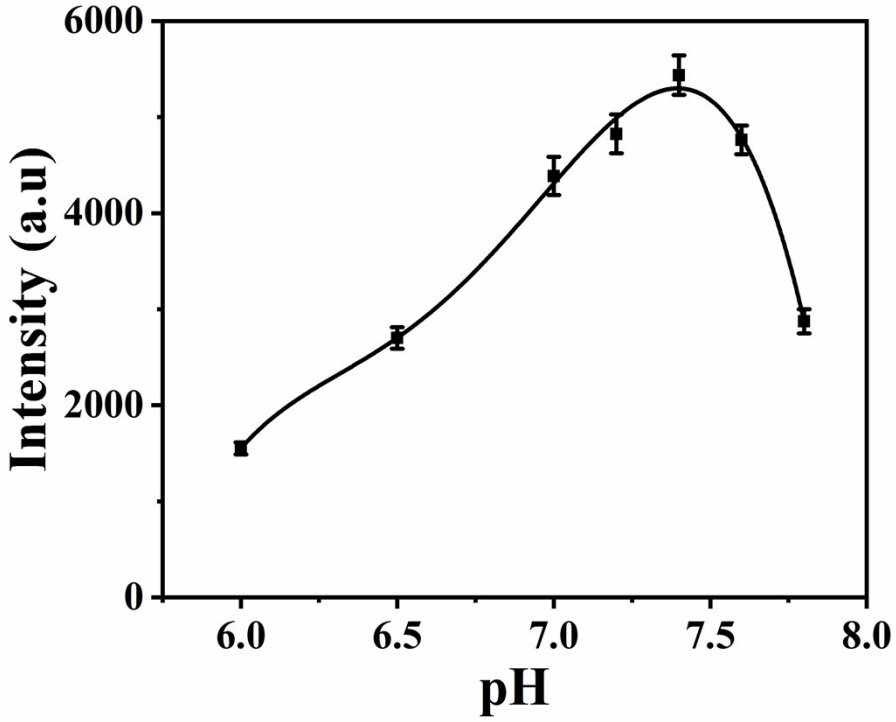

Fig. S4 SERS intensities of NBA at 592 cm^{-1} for caspase-3 detection recorded from PBS buffer with different pH (6.0, 6.5, 7.0, 7.2, 7.4, 7.6, and 7.8).

1.4 Selectivity and stability evaluation of SERS probes

The specific biomarker (caspase-3) as well as nonspecific caspases (caspase-2, caspase-4 and caspase-9) with the same concentration (10 fM) in PBS buffer were further detected, respectively. The peak intensities of NBA at 592 cm^{-1} were employed as the characteristic peaks of caspase-3. Fig. S5A and Fig. S5B showed that SERS spectra and corresponding SERS intensities of NBA at 592 cm^{-1} for specific analyses of caspase-3 and nonspecific caspases, respectively. The significantly stronger SERS signal was observed with caspase-3 than those of nonspecific caspases, indicating that there was no significant interference from most of the non-specific caspases tested to the biosensor in our experiments. All these results indicated that the biosensor exhibited an acceptable selectivity for the determination of caspase-3.

The effect of pH on the response of this Au nanoboxes-NBA-peptide probes was tested. As shown in Fig. S6, the peak intensities of NBA at 592 cm^{-1} stayed almost constant when pH was increased from 6.0 to 8.0. The result indicated that this probe possessed high stability against potential interferences from pH change.

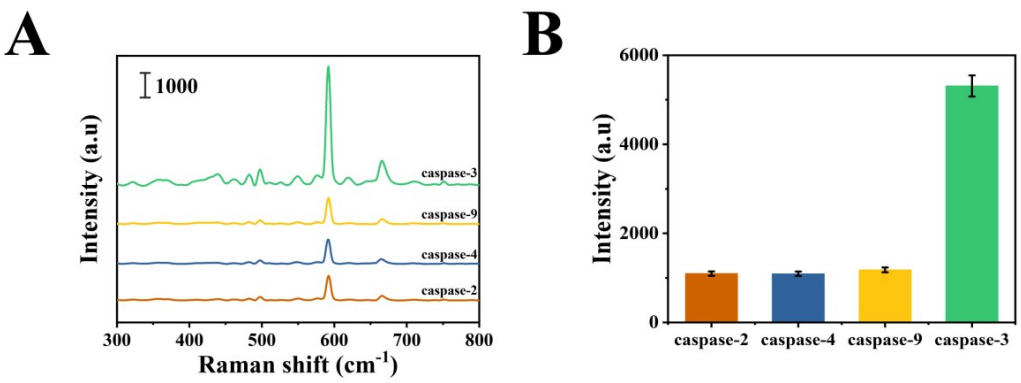

Fig. S5 (A) SERS spectra and (B) corresponding SERS intensities of NBA at 592 cm^{-1} for specific analyses of caspase-3 and nonspecific caspases. The error bar represents the standard deviation of different batches (n=3). The concentrations of caspase-3 and noncomplementary caspases were 10 fM.

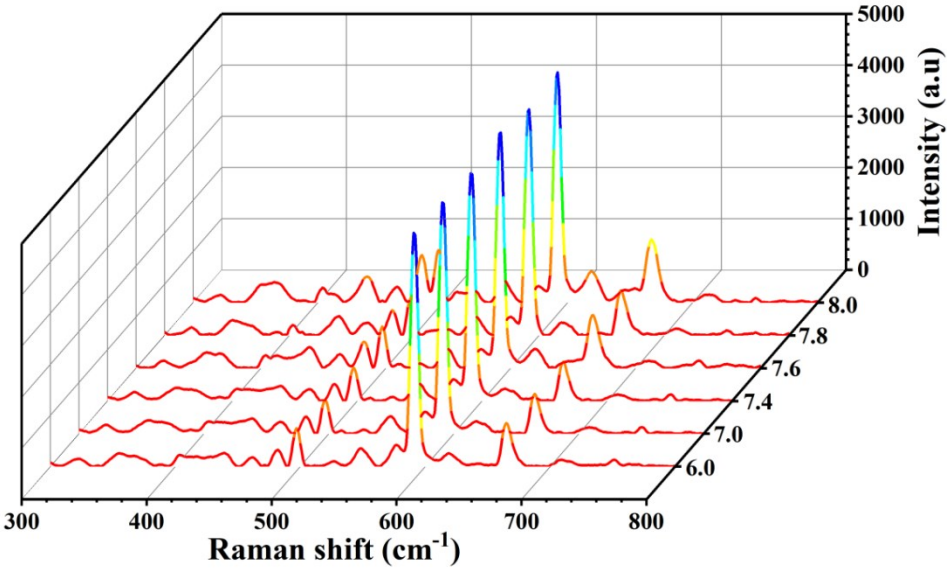

Fig. S6 SERS spectra of Au nanoboxes-NBA-peptide probes recorded from PBS buffer with different pH (6.0, 7.0, 7.4, 7.6, 7.8, and 8.0).

1.5 Dynamic behavior of Au nanoboxes-NBA-peptide probes during incubation

In the presence of trace intracellular caspase-3, the substrate peptide can be cleaved and the changed surface charge of the Au nanoboxes results in the Au nanoboxes-NBA-peptide assembled to form aggregation and the great enhancement of SERS intensity. It could be observed from Fig. S7 that probes were constantly aggregating

with the extension of incubation time from 1 h to 4 h and then plateaued at 5 h. This was consistent with the changes in SERS intensities at 592 cm^{-1} of HeLa cells incubated with Au nanoboxes-NBA-peptide probes from 1 h to 5 h. Therefore, 4 h was selected as the optimal reaction time.

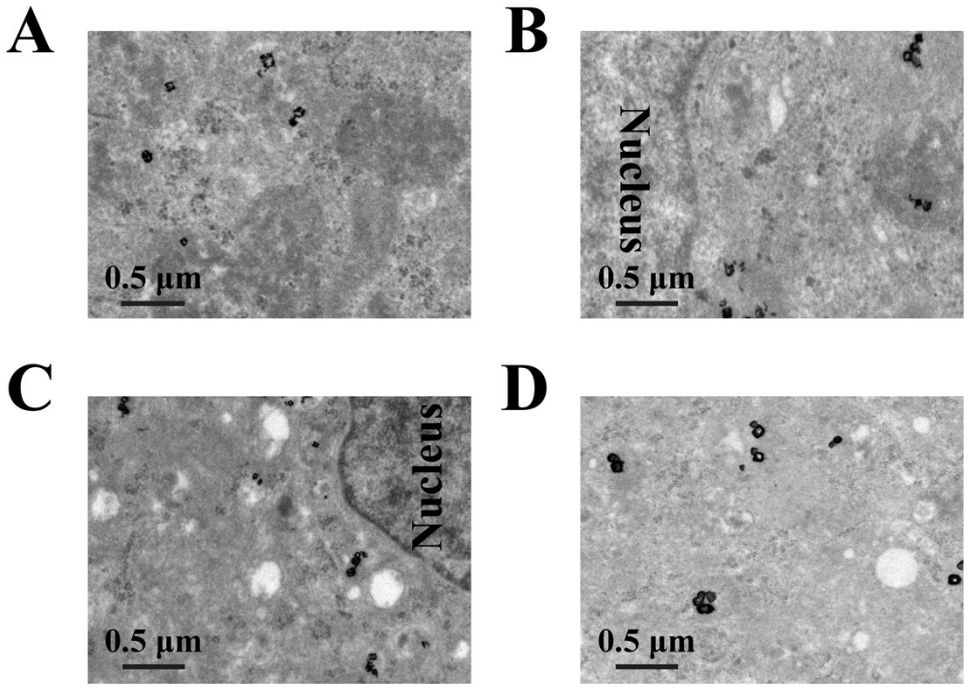

Fig. S7 TEM images of Au nanoboxes-NBA-peptide SERS probes inside the HeLa cells at (A) 1 h, (B) 2 h, (C) 4 h, and (D) 5 h of incubation.

1.6 Comparison of different methods used for caspase-3 detection

Table S1 was the comparison of different methods used for the detection of caspase-3. Generally, the LOD of the proposed method was lower than most reported methods, indicating that the proposed strategy was more sensitive than the reported techniques.[3-10]

Table S1 Comparison of different methods used for the detection of caspase-3.

Method	Limit of detection (LOD)	Ref.
Fluorescence (BRET-FRET)	0.41 nM	3
Fluorescence	0.4 nM	4
Fluorescence	3.2 nM	5
FRET	36.4 aM	6
Chemiluminescence	20 pM	7
Colorimetry	0.89 nM	8
ICP-MS	0.31 nM	9
Square Wave Voltammetry	100 pM	10
SERS	0.127 fM	This work

References

1 J. W. Qi, Y. D. Li, M. Yang, Q. Wu, Z. Q. Chen, W. D. Wang, W. Q. Lu, X. Y. Yu, J. J. Xu and Q. Sun, *Nanoscale Res. Lett.*, 2013, **8**, 1-6.

2 M. A. Mahmoud and M. A. El-Sayed, *J. Am. Chem. Soc.*, 2010, **132**, 12704-12710.

3 B. R. Branchini, J. C. Rosenberg, D. M. Ablamsky, K. P. Taylor, T. L. Southworth and S. J. Linder, *Anal. Biochem.*, 2011, **414**, 239-245.

4 H. B. Wang, Q. Zhang, X. Chu, T. T. Chen, J. Ge and R. Q. Yu, *Angew. Chem., Int. Ed.*, 2011, **50**, 7065-7069.

5 Z. Gu, A. Biswas, K. I. Joo, B. L. Hu, P. Wang and Y. Tang, *Chem. Commun.*, 2010, **46**, 6467-6469.

6 L. Liu, H. Zhang, D. Q. Song and Z. X. Wang, *Analyst*, 2018, **143**, 761-767.

7 X. Huang, Y. Liang, L. Ruan and J. Ren, *Anal. Bioanal. Chem.*, 2014, **406**, 5677-5684.

8 Z. Zhou, L. Peng, X. Wang, Y. Xiang and A. Tong, *Analyst*, 2014, **139**, 1178-1183.

9 Z. R. Liu, B. B. Chen, M. He, X. Zhang, H. Wang and B. Hu, *Analyst*, 2016, **141**, 926-933.

10 B. Khalilzadeh, N. Shadjou, M. Eskandani, H. N. Charoudeh, Y. Omidi and M. R. Rashidi, *RSC Adv.*, 2015, **5**, 58316-58326.