Supporting information

Enzyme-triggered click chemistry combined with surface-enhanced Raman spectroscopy for simple and sensitive detection of alkaline phosphatase activity from complex biological samples

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1 Materials and Methods

1.1 Materials and instruments

Silver nitrate ($AgNO_3$, > 99.8%), Trisodium citrate dehydrate (99%) and Copper sulfate were purchased from Shanghai Chemical Reagent Co., Ltd. Azide terephthalic acid ,4-acetylene biphenyl and sodium orthovanadate (Na_3VO_4) were purchased from Aladdin Industrial Corporation (Shanghai, China). Alkaline phosphatase (ALP), ascorbic acid-phosphate, ascorbic acid, phosphodiesterase (PDE), acid phosphatase (ACP), trypsin and lysozyme were obtained from Sigma-Aldrich.

The morphologies of the AgNPs were characterized by the transmission electron microscope (TEM, Hitachi 600). The UV-vis absorption spectra of AgNPs was obtained with a Lambda 750 spectrophotometer (Perkin-Elmer). SERS measurements were carried out at 632.8 nm from a laser power of ~7 mW and an accumulation time of 5 s per time.

1.2 ALP-triggered click reaction

The AgNPs acted as SERS enhanced substrate were synthesized by the citrate reduction reaction according to Lee's method.[1] Briefly, 0.018 g of $AgNO_3$ was dissolved in 100 mL of deionized water and brought to boiling. Then, 1.0 mL of 1% trisodium citrate aqueous solution was added, and the mixture was kept boiling for 50 min. The transmission electron microscopic (TEM) image shows they are all in a quasi-spherical shape with an average size of 55 nm (Fig. 1a&S1).

The azide terephthalic acid (1 mM) and 4-acetylene biphenyl (1 mM) were mixed at a volume ratio of 1:1 before use for all the experiments. Then, the solution mixture

including 20 μL of ALP (50 U/L), 20 μL of ascorbic acid-phosphate (20 mM), and 60 μL of Tris-HCl (1 mM) were kept to react at 35℃ for 1 h. After that, 20 μL of the mixture was added to the centrifuge tube that contained 20 μL of Cu^{2+} (500 μM) and 160 μL of azide terephthalic acid/4-acetylene biphenyl for the ALP-triggered click reaction. After 10 min, 50 μL of the above mixed solution were added into 1 mL of silver nanoparticle and stand for 10 min at room temperature, and then the SERS spectra of the 4-AB was recorded. The assay without ALP in the above process is used as a control group.

1.3 ALP activity monitoring using the SERS-click strategy

ALP was serially diluted with 1 mM Tris-HCl buffer to reach a final concentration ranging from 0.2 U/L to 50 U/L. 20 μL of different ALP activity (0.2-50 U/L), 20 μL of ascorbic acid-phosphate (20 mM), and 60 μL of Tris-HCl (1 mM) were kept to react at 35℃ for 1 h, respectively. The procedures of click reaction described above were repeated. Then, 10 μL of the reacted solution was dropped on the glass slide, and the SERS spectra of 4-AB were measured after they were dried.

We selected other enzymes, such as, trypsin, phosphodiesterase (PDE), acid phosphatase (ACP) and lysozyme to investigate the selectivity of the SERS-click method to ALP. Under the optimal conditions that pH 7.4, temperature 35℃, the activity of the interference enzymes (50 U/L) including PDE, ACP, trypsin and lysozyme were analysed. The peak intensities of 4-AB at 1596 cm^{-1} were obtained from the SERS spectra of all the assay groups and they were plotted for comparison.

1.4 Determination of ALP activity in serum and cell lysate

To prepare cell lysate, HepG2 cells were cultured in DMEM supplemented with 10% fetal bovine serum at 37℃ with 5% CO_2. After aspirating the DMEM, the HepG2 cells were washed twice with 2 mL of cold PBS buffer. RIPA lysate buffer (450 mL) was added into the plate and incubated for 12 min on ice. Subsequently the suspended cells were transferred to a 2 mL tube and then passed through a 25 gauge needle 12 times to extract cell lysates. The HepG2 cell lysates were incubated on ice for another 12 min and centrifuged at 4℃ (12000 rpm, 10 min). Finally, the supernatant was transferred to a 1.5 mL tube on ice for subsequent SERS measurement.

For ALP activity determination, Cell lysate stock solution was diluted to 0.1 mg/mL in 100 mL of Tris-HCl buffer (pH 7.4). The mixed solution including ascorbic acid-phosphate (20 mM), Cu^{2+} (500 μM) and azide terephthalic acid/4-acetylene biphenyl (1 mM) were added to the solution. After 70 min of incubation at 35℃, the SERS spectra were obtained with an accumulation time of 5 s per time.

1.5 Evaluation of ALP inhibitor

To evaluate the inhibition efficiency of Na_3VO_4 toward ALP, 10 μL of Na_3VO_4 with different concentrations (0, 20, 100, 150, 300, 500 and 800 μM) were added into the aqueous solution of ALP (10 μL, 50 U/L) at 37℃ for 40 min. Then the Na_3VO_4-treated ALP was added into the mixed solution containing ascorbic acid-phosphate (20 mM), Cu^{2+} (500 μM) and azide terephthalic acid/4-acetylene biphenyl (1 mM). The resulting mixtures were incubated at 35℃ for 70 min for evaluating the inhibition efficiency of Na_3VO_4 toward ALP.

2 The statistic particle sizes of the prepared AgNPs

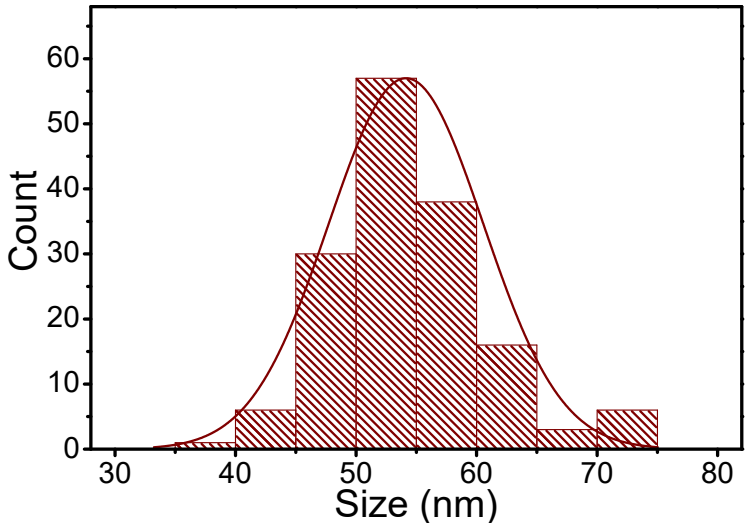

Fig. S1 The statistic particle sizes of the prepared AgNPs.

According to the above statistical results, the average particle diameter of the AgNPs obtained by TEM image was 55±3.5 nm. In order to further verify the particle size of the AgNPs prepared by the sodium citrate reduction method, we performed dynamic light scattering experiments to determine the particle size distribution of the nanoparticles. The results show that the particle size range of AgNPs is 49±7.4 nm.

3 Characterization of click reaction

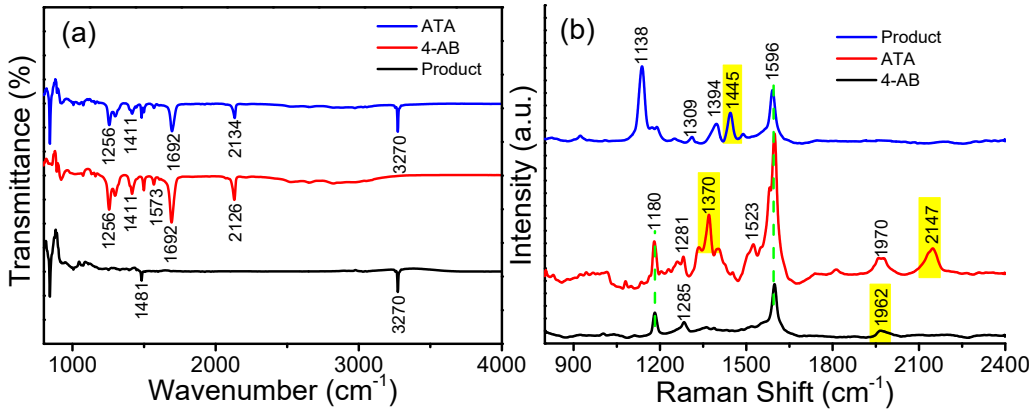

Fig. S2 Comparison of (a) Fourier transform infrared spectroscopy (FTIR) and (b) Raman spectra of 4-AB, ATA and click reaction product.

4 Reproducibility of SERS detection

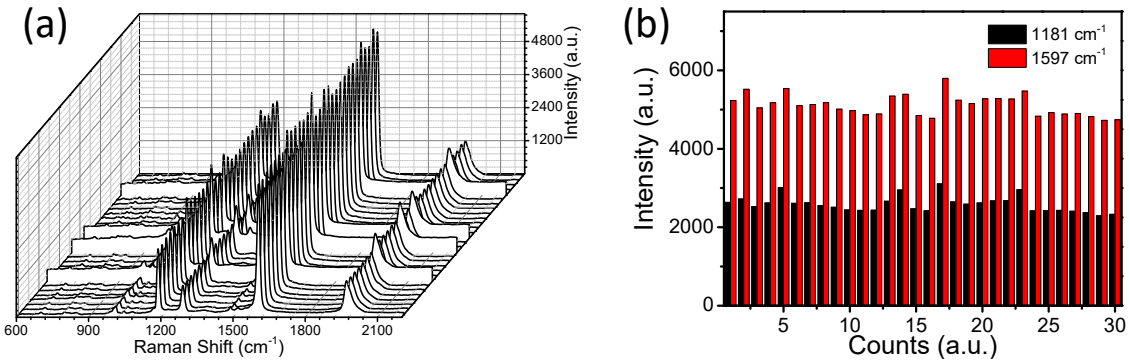

Fig. S3 (a) SERS spectra from randomly selected 30 points on the same sample. (b) The intensities of SERS peaks at 1181 cm^{-1} and 1597 cm^{-1} obtained from data (a).

To evaluate the reproducibility of this SERS-click strategy for monitoring ALP activity, we have randomly selected 30 point on the same sample to compare the SERS signal of 4-AB in Fig. S3. Two peaks at 1181 and 1597 cm^{-1} were chosen to evaluate its reproducibility and we found that the relative standard deviations (RSD) of Raman signals are calculated as 3.41 % and 5.36 % in Fig. S3b, which can meet the requirement on the RSD of less than 20 %.[2] These data indicate that this SERS-click strategy present acceptable reproducibility for detecting ALP.

Table.S1 The assignments of the Raman spectra of 4-AB, ATA and click reaction product.

Substance	Raman Shift (cm^{-1})	Assignment
4-acetylene biphenyl (4-AB)	1002	Benzene out-of-plane
	1180	Benzene ring stretch (7a)
	1596	Benzene ring stretch (8a)
	1962	C≡C stretching mode
Azide terephthalic acid (ATA)	1180	Benzene ring stretch (7a)
	1370	Symmetric stretching vibration of N3
	1596	Benzene ring stretch (8a)

	2147	Antisymmetric stretching vibration of N3
Click reaction product	1138	Ring breathing vibration
	1394	C-C stretching vibration
	1445	stretching vibration of N=N
	1596	Benzene ring stretch (8a)

Table.S2 The assignments of the FTIR spectra of ATA, 4-AB and click reaction product.

Substance	Wavenumber (cm^{-1})	Vibration mode
ATA	1256	C-H stretching vibration
	1411	C-H in-plane bending vibration
	1692	C=C skeleton vibration mode
	2134	Anti-symmetric stretching vibration of N3
	3270	O-H stretching vibration of COOH-Ar
4-AB	1256	C-H stretching vibration
	1411	C-H in-plane bending vibration
	1573	C=C stretching vibration
	1692	C=C skeleton vibration mode
	2126	C≡C stretching vibration mode
Product	1481	stretching vibration of N=N
	3270	O-H stretching vibration of COOH-Ar

Table.S3 Comparisons of the proposed ALP strategy with other reported methods.

Method	Limit of Detection (U/L)	Detection Range (U/L)	R^2	Refs.
fluorescence	8.0	20-1000	--	[3]
electrochemistry	5	5-640	0.9981	[4]
fluorescence	0.3	0.3-7.5	0.9938	[5]
electrochemistry	0.4	5-1000	--	[6]
colorimetry	10	100-600	0.9931	[7]

SERS-click strategy	0.2	0.2-50	0.921	Ours

Table.S4 The recovery rate of ALP in the serum. Each sample was measured five times.

Sample No.	Real value (U/L)	Measured value (U/L)	Recovery (%)
1	6	6.17±0.4	102.83±6.67
2	10	10.82±0.312	108.2±3.12
3	15	15.24±0.292	101.6±1.95
4	20	17.88±0.4	89.4±2

References

[1] P. C. Lee and D. J. Meisel, *Phys. Chem*., 1982, **86**, 3391-3395.

[2] M. J. Natan, *Faraday Discuss*., 2006, **132**, 321-328.

[3] M. C. L. Yeung and V. W. W. Yam, *Chem. Sci*., 2013, **4**, 2928-2935.

[4] A. Hayat and S. Andreescu, *Anal. Chem*., 2013, **85**, 10028-10032.

[5] L. Zhang, J. Zhao, M. Duan, H. Zhang, J. Jiang and R. Yu, *Anal. Chem*., 2013, **85**, 3797-3801.

[6] Z. M. Yang, G. L. Liang, M. L. Ma, Y. Gao and B. Xu, *Small*, 2007, **3**, 558-562.

[7] C. M. Li, S. J. Zhen, J. Wang, Y. F. Li and C. Z. Huang, *Biosens. Bioelectron*., 2013, **43**, 366-371.