Electronic Supplementary Information

A Sensitive SERS Assay of L-histidine via DNAzyme-activated Target Recycling Cascade Amplification Strategy

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Experimental section:

Reagents: All chemicals and materials were purchased at their purest grade and used as received. Oligonucleotides designed in this study were purchased from SBS Genetech. Co. Ltd. (Shanghai,China). The carboxyl-modified MBs (1.0-2.0 μm) were purchased from Tianjin Baseline ChromTech Research Centre (China). The deoxynucleotide solution mixture (dNTPs) and polymerase Klenow Fragment exo-(10 u/ul) accompanied by 10 Klenow Fragment exo-buffer (50 mM KPB buffer (pH 5.6),1 mM DTT,Glycerol 50%) were purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. L-histidine was purchased from (supplied) Shanghai jie Rachel biological engineering Co., LTD. Solutions were prepared with Milli-Q deionized water. The pH of all buffers was adjusted with either NaOH or HCl solution. All the chemicals are of analytical-reagent grade and without further purification. Deionized water was used throughout the experiments.

Apparatus: UV/Vis absorption spectra were obtained with a Cary 50 Series Spectrophotometer (Varian, Australia). Raman measurements were conducted in a Renisaw inVia Raman spectrometer (RamLab-010) at an excitation laser of 633 nm. A microscope equipped with a 50 × objective was used to focus the incident excitation laser. The laser power on the sample was 5 mW, and the accumulation time was 10 s, the Raman spectra were calibrated with the WiRE Raman Software Version 2.0 from Renisaw Ltd. Transmission elec-tron microscopy (TEM) images were recorded on a JEM 1200EX trans-mission electron microscope (JEOL, Japan).

The immobilization of the hairpin probe: After washed three times with imidazole-HCL, 10 μL of carboxyl-modified MBs were activated in 200 μL of imidazole buffer (0.1 M, pH 6.8) containing EDC (0.1 M) with gentle shaking for 30 min. Then the hairpin DNA (100 nM, 10 μL) was added and incubated at 37 °C with extensive stirring for 12 h.

Preparation of Au nanoparticles (AuNPs): AuNPs were prepared by the citrate reduction method[2]. Trisodium citrate (2 mL, 1%) was added to boiling $HAuCl_4$ solution (50 mL, 0.01%) and stirred for 20 min at the boiling point. When the color of the solution turned to deep red from gray yellow, it could indicate the formation of AuNPs. Then it was cooled naturally to room temperature. The average diameter of AuNPs was about 20 nm. The prepared gold colloidal solution was stored in brown glass at 4 °C in the refrigerator.

Preparation of bio-bar code DNA probe: 1 mL of Au colloids was transferred into a 2 mL Eppendorf tube. 50 μL 1×10^{-7} M of 5'-thiol signal DNA and 10 μL 1×10^{-8} M capture DNA (the ratio of capture DNA to bio-bar code rhodamine- modified DNA is 1:50[3]) were added to 1 mL of the prepared gold nanoparticles and then incubated at 37 °C for 18 h. The DNA/Au NPs conjugates were aged in salts (0.05 mol NaCL, 200 μL) for 6 h and salts (0.1 mol NaCl, 200 μL) for 6 h, respectively. Excess reagents were removed by centrifuging at 10,000 rpm for 30 min. Finally, the resulting precipitate was dispersed into PBS buffer (100 μL).

The sensing procedure: The aptamer probe to L-histidine and the hairpin probe were transferred into a new Eppendorf tube. Then the bio-bar code DNA (10 μL), the primer DNA (15 μL), the polymerase Klenow (0.3 $U\mu L^{-1}$), dNTPs (6 μL), different

concentrations of L-histidine were added into the system successively. After reacting 1.5 h by gentle shaking, the resulting solution was detected by Raman spectrometer.

SERS Measurements: The MBs incorporated with SERS probes were washed with PBS for three times and redispersed in 100 μL of 0.01 M PBS buffer (pH 7.4). The excess bio barcodes were removed by magnetic separation. After we casted 3 μL of each solution onto the surface of Au slide, the Au slide was air-dried at room temperature ahead of SERS analysis. The SERS spectra were measured by an inVia Raman microscope with a 633 nm laser. The laser power was 5 mW, and the acquisition time for each spectrum was 10 s. Three spectra from different sites were collected from each sample and averaged to represent the SERS results, and three repeated experiments were performed. Error bars showed the standard deviation of three experiments.

Table S1 DNA sequence used in this work.

oligonucleotides name	sequences	discription
DNAzyme sequence	5′- AGG TAA TTC GTA CAC **rA** GG AAG AGA TG GC T (8) GC CAT CTC TTA ACG GGG CTG TGC GGC TAG GAA GTA GTG TAC GAA C-3′	Red part means RNA base.
S1 sequence	5′-GTA CAC TAC TTC-3′	Primer DNA complementary to DNAzyme
hairpin DNA	5′- NH_2- TTT TTT TCG TAG TCC TTG TGT ACG AAT TAC CTC AGC AGG ACT ACGT-3′	Hairpin DNA immobilized on MB
signal DNA	5-Rox-TTT TTT CCT AGC GAC-SH-3	The signal probes immobized on the Au nanoparticle
capture probe	5-SH-TTT TTT ACG TAG TCC-3	The capture probe immobized on the Au nanoparticle

UV-visible spectra of the Rox-DNA conjugates: In Figure S1, the prepared SERS probes were characterized by a Cary spectrometer. The curve of a, c, d respectively exhibited the characteristic absorbance, curve a of Au NPs (~ 520 nm), curve c of Rox-DNA (~260 nm and two characteristic absorbance at 500~600 nm) according to the literature[4] and curve d of DNA (~260 nm). The appearance of characteristic absorbance of Au NPs at ~520 nm as exhibited in Curve b indicated that Rox-DNA had successfully fabricated with Au NPs on the 3'- terminus.

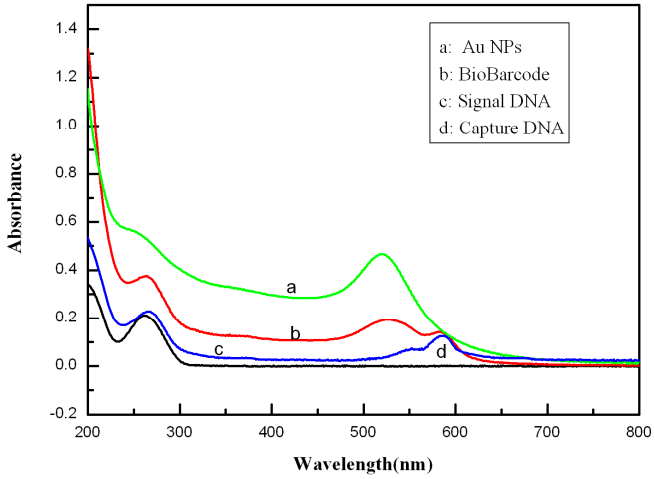

Figure S1. UV spectra of Au colloid (a), bio barcode DNA (b), Rox-DNA (c) and capture DNA(d);

Characterization of AuNPs:

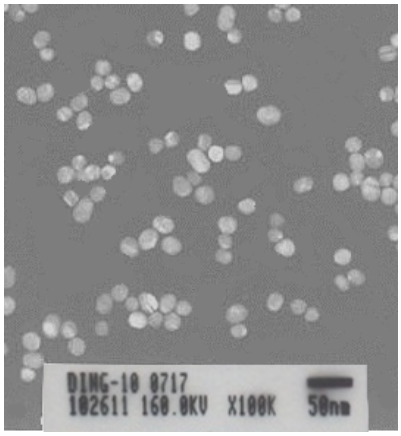

Figure S2.The TEM of Au NPs (~20 nm).

Optimization of pH of PBS buffer solution: The effect of pH of PBS buffer solution is shown in Figure S3. As we all know, pH of PBS buffer solution plays an important role in the cycle reaction, so we investigated the intensity of Raman detection in a pH range of 6.0-9.0. As shown in Figure S3, the intensity of Raman detection reached the maximum at pH 7.9. Thus, the pH 7.9 was selecteded for the optimum condition.

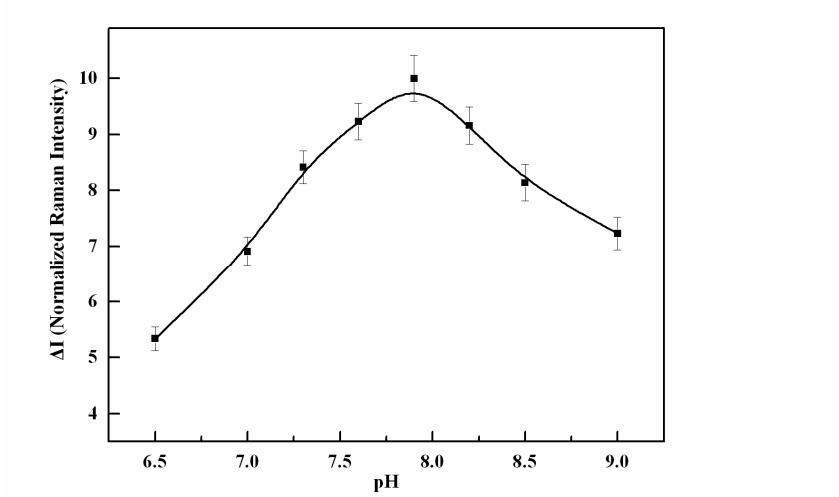

Figure S3. Effect of the pH of the recycling reaction on the ΔI signal, L-histidine concentration, 50 nM. SERS spectra were measured with excitation at 633 nm, 5 mW laser power and the acquisition time for each spectrum was 10 s.

Optimization of reaction temperature: the reaction temperature has a great effect on the DNAenzyme bioactivity, so we investigated the intensity of Raman detection under different temperature conditions. In the examined temperature range, the maximum intensity occurred at 37 °C. Taking the DNAenzyme bioactivity into account, 37 °C was used as the reaction condition.

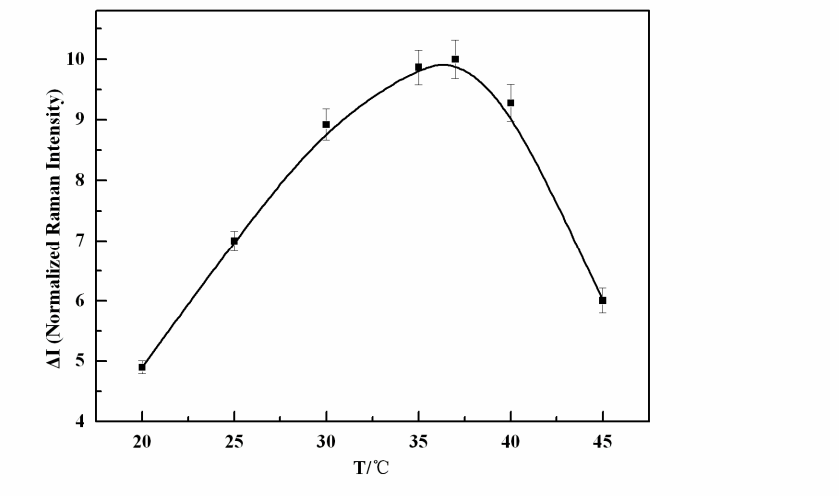

Figure S4. Effect of the temperature of the recycling reaction on the ΔI signal, L-histidine concentration, 50 nM. SERS spectra were measured with excitation at 633 nm, 5 mW laser power and the acquisition time for each spectrum was 10 s.

Optimization of the amount of Klenow polymerase: To respectively investigate the influence of the amount of Klenow polymerase, which was used in the experiment of L-histidine detection, the Raman signals produced by 50 nM L-histidine were measured by using various amounts of polymerase and nicking enzyme. As shown in Figure S5, when the volume of polymerase increased from 0.05 to 0.50 $U\mu L^{-1}$, the Raman intensity increased gradually. But after that, the Raman intensity

tended to reach a platform. Therefore, 0.3 $U\mu L^{-1}$ of Klenow polymerase was considered to be the optimum amount used in the L-histidine analyzing reaction.

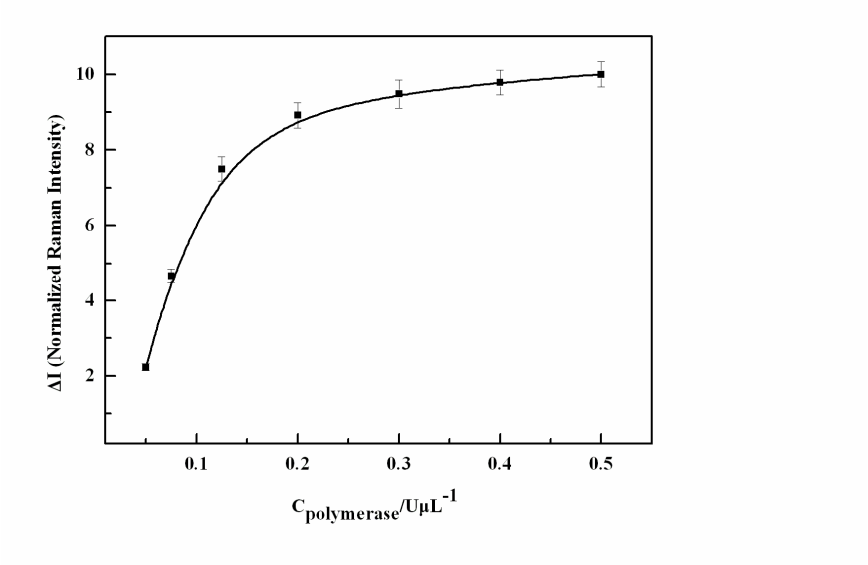

Figure S5. Effect of the amount of polymerase on the ΔI signal, L-histidine concentration, 50 nM. SERS spectra were measured with excitation at 633 nm, 5 mW laser power and the acquisition time for each spectrum was 10 s.

Optimization of the incubation time: In order to achieve the system' best sensing performance, the incubation time was optimized, as shown in Figure S6. The incubation time for polymerization and DNAzyme-catalyzed cleavage reaction is a critical element to the system because it initiates a circulating signal transformation process. According to Figure S6, the detection of Raman intensity was determined upon different incubation times ranging from 0 min to 150 min. Once the cycle was triggered, the Raman intensity nearly reached a plateau after 90 min. Finally, 90 min were regarded as the optimum incubation time.

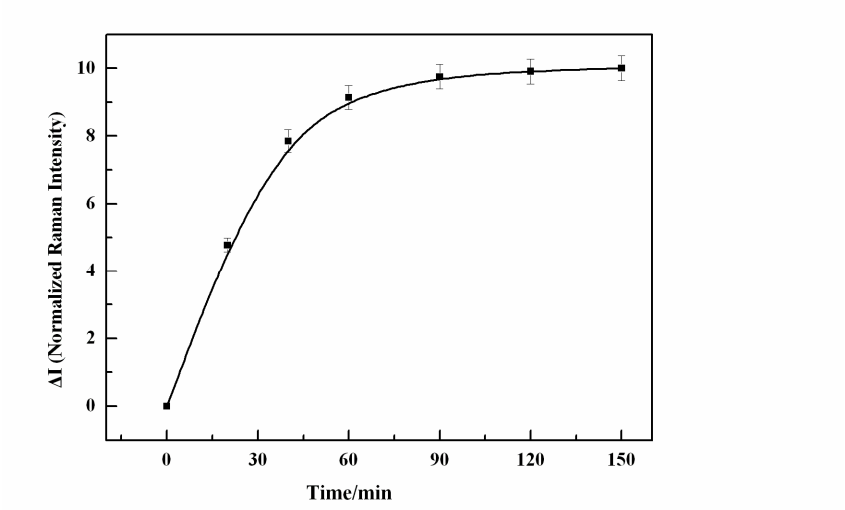

Figure S6. Effect of the time of the recycling reaction, L-histidine concentration, 50 nM. SERS spectra were measured with excitation at 633 nm, 5 mW laser power and the acquisition time for each spectrum was 10 s.

Table S2. Comparison of different methods for L-histidine detection.

Method	Transducer	Detection limit	The description of the following methods
DNAzyme-based biosensor with recycling amplification	fluorescence	200 nM[5]	An efficient fluorescent biosensor was designed by DNAzyme-based fluorescent beacons via a endonuclease-assisted recycling cleavage strategy.
Gold nanoparticles grapheme nanosheets -based sensor	electrochemistry	0.1 pM[6]	Gold nanoparticles–grapheme nanosheets (GNPs-GNSs) composite was prepared as the SERS substrate.
Ni^{2+}-modified gold nanoclusters based fluorescence detection	fluorescence	30 nM[7]	The Ni^{2+}-modified gold nanoclusters for fluorescence turn-on detection of histidine is fabricated, based on the high affinity of L-histidine to Ni^{2+}.
The cator-displacement assay	colorimetry	0.4 μM[8]	Murexide, was used as the indicator and the selective detection of histidine was achieved based on the competition between indicator and histidine for the binding with Ni^{2+}.
DNAzyme-based assay with enzyme amplification	chemiluminescence	500 μM[9]	DNAzyme activated by L-histidine-dependent DNAzymes yield the horseradish peroxidase-mimicking catalytic nucleic acids that enable the colorimetric detection of L-histidine.
This method	SERS	0.56 nM	Firstly, the detection limit is much lower than previously reported L-histidine sensors[5, 7-9]. Moreover, This method is designed with polymerase-assisted recycling, avoiding the increase background value with the introduction of endonuclease [5]. Secondly, compared with GNPs-GNSs-based sensor[6]., this method does not need complicated preparation of the SERS substrate and has a higher selectivity with more potential interferences in spite of the lower sensitivity.

Specificity of the method for L-histidine detection:

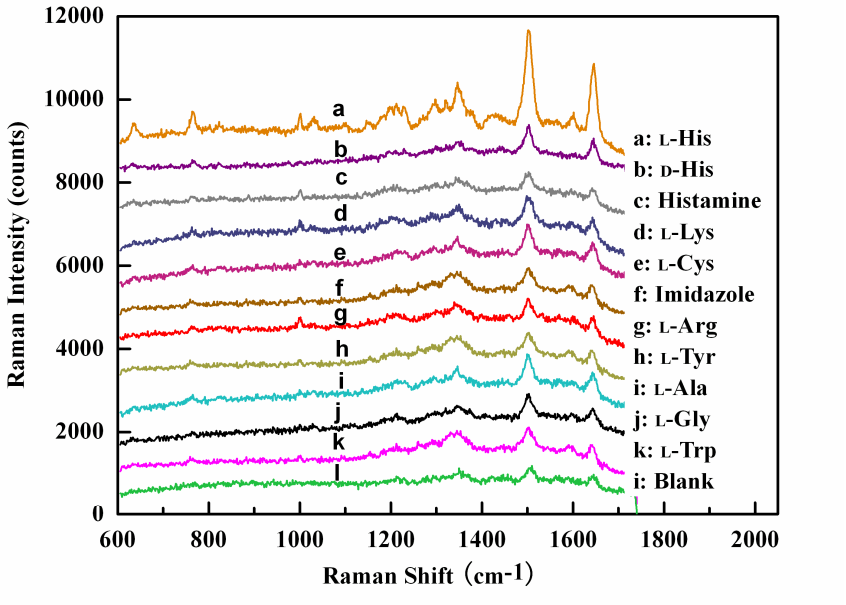

Figure S7. SERS spectra for the detection of of L-histidine over other potential interferences (L-histidine at concentration of 50 nM and all other compounds at concentration of 0.5 μM). SERS spectra were measured with excitation at 633 nm, 5 mW laser power and the acquisition time for each spectrum was 10 s.

Determination of L-histidine in real samples: To further demonstrate that the proposed method could be used in the detection of practical samples, we analyzed varying amounts of L-histidine in a different background solution by adding a certain amount of cellular homogenate (1.0×10^5 cells/mL) which contained a very large number of different proteins and contaminants. As shown in Table S3, the recoveries of spiked histidine ranged from 89.2% to 94.2%, confirming that the SERS sensing system was applicable for practical L-histidine detection in real samples with other potentially competing species coexisting.

Table S3. Detection of L-histidine in cellular homogenate.

Nos.	L-histidine content added (M)	L-histidine content detected (M)	recovery (%)
1	5×10^{-9}	4.46×10^{-9}	89.2%
2	10^{-8}	9.02×10^{-9}	90.2%
3	5×10^{-8}	4.71×10^{-8}	94.2%

Refrences:

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