# Electronic Supplementary Materials (ESM)

# Enhanced fluorometric detection of histamine using red emissive amino acid-functionalized bimetallic nanoclusters

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## Instrumentation and calculation of quantum yield

Fluorescence emission spectrum measurements were conducted using a Shimadzu RF-5301PC fluorescence spectrometer (Tokyo, Japan). The instrument utilized a 1 cm quartz cell and employed a 5 nm slit width. UV-Vis measurements were performed using a Shimadzu UV-1601 spectrophotometer (Tokyo, Japan). Transmission electron microscopy (TEM) images were captured using a JEOL JEM-1400 microscope operating at 200 kV (Japan). FT-IR spectral studies were carried out using a Nicolet 6700 FT-IR series spectrophotometer with KBr pellets as the source material, covering a spectral range from 4000 $cm^{-1}$ to 400 $cm^{-1}$. X-ray diffraction patterns were obtained using a Philips PW 1700 X-Ray diffractometer (Eindhoven, Netherlands). X-ray photoelectron spectroscopy (XPS) data were acquired using an ESCALAB 250 XI instrument (Thermo Scientific) equipped with Al Kα X-ray radiation as the excitation source. Particle size measurements were conducted using a Zetasizer nano analyzer. Dynamic light scattering (DLS) measurements were conducted using the Zetasizer Red Badge instrument of the ZEN 3600 Nano ZS model from Malvern, UK. Elemental analysis (EDX) for demonstrating the elemental composition of LYS@Ag/Au NCs was carried out with the NEX QC+ QuantEZ.

The quantum yield (Ø) of LYS@Ag/Au NCs was calculated using quinine sulfate (0.1 M $H_2SO_4$ as solvent) as a standard reference, i.e., at the excitation wavelength of 350 nm, its QY was 56% in 0.1 M $H_2SO_4$ solution. The fluorescent spectra of quinine sulfate and LYS@Ag/Au NCs were measured at excitation wavelengths of 350 nm and the absorbance was kept under 0.05. The quantum yield was calculated according to the following Equation:

$$\phi = \phi_{quinine} \times \frac{F_{LYS@Ag/Au\ NCs}}{F_{quinine}} \times \frac{A_{quinine}}{A_{LYS@Ag/Au\ NCs}} \times \frac{\eta_{LYS@Ag/Au\ NCs}}{\eta_{quinine}} \quad \text{II}$$

Ø denotes the QY of LYS@Ag/Au NCs; F and A are the integral area of fluorescence emission peak and UV–Vis absorbance intensity at excitation wavelength, respectively; η is the refractive index of the solvent.

## Assay of real samples

### Human serum

Fixed volume of human serum (450 µL without and spiked with histamine) was mixed with 300 µL of LYS@Ag/Au NCs and incubated at room temperature for 3 minutes. Subsequently, the volume was adjusted to 1.0 mL using ultrapure water. Finally, the fluorescence responses were measured at 640 nm after excitation at 360 nm.

### Canned tuna fish

The canned tuna fish was thoroughly homogenized, and 2.5 g of the homogenized sample was transferred to a 50 mL Falcon tube. Each sample was then spiked with different concentrations of histamine and vortexed for 100 seconds to ensure complete mixing. Next, 6 mL of methanol was added to the sample mixture, followed by another 100 seconds of vortexing. The samples were then subjected to centrifugation at 3500 rpm for 15 minutes. The resulting mixture was filtered, and the clear extract was treated with 10 mL of hexane. The hexane layer was vortexed for 100 seconds and then centrifuged to remove fatty acids. The hexane layer was discarded, and the aqueous layer was retained for further analysis. For the subsequent analysis, 450 µL of the aqueous layer was mixed with 300 µL of LYS@Ag/Au NCs and incubated at room temperature for 3 minutes. The volume was then adjusted to 1.0 mL using ultrapure water. Finally, the fluorescence responses were measured at 640 nm after excitation at 360 nm.

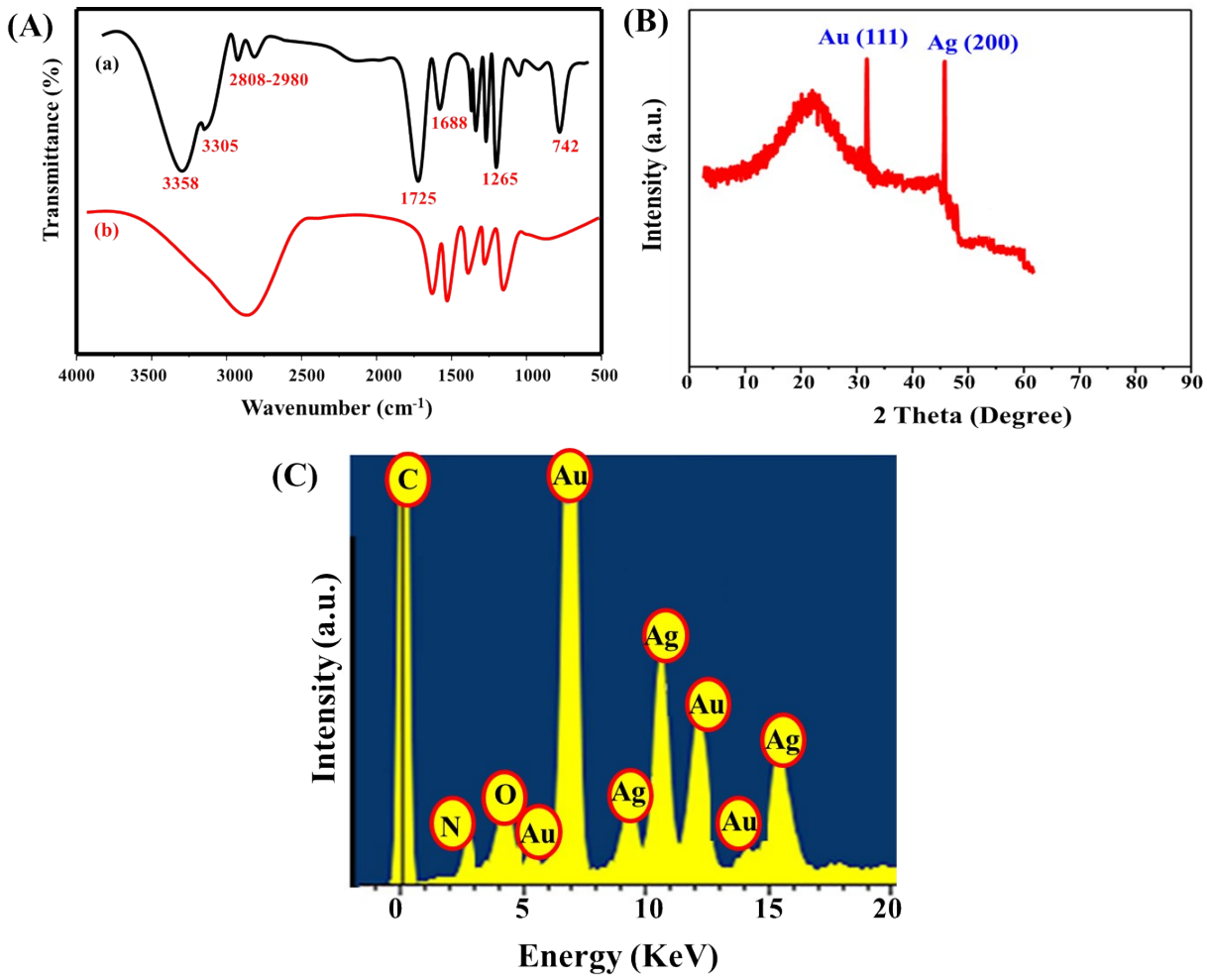


**Fig.S1** (A) FTIR of LYS (a) and LYS@Ag/Au NCs (b); (B) XRD of LYS@Ag/Au NCs; (C) EDX pattern of LYS@Ag/Au NCs.

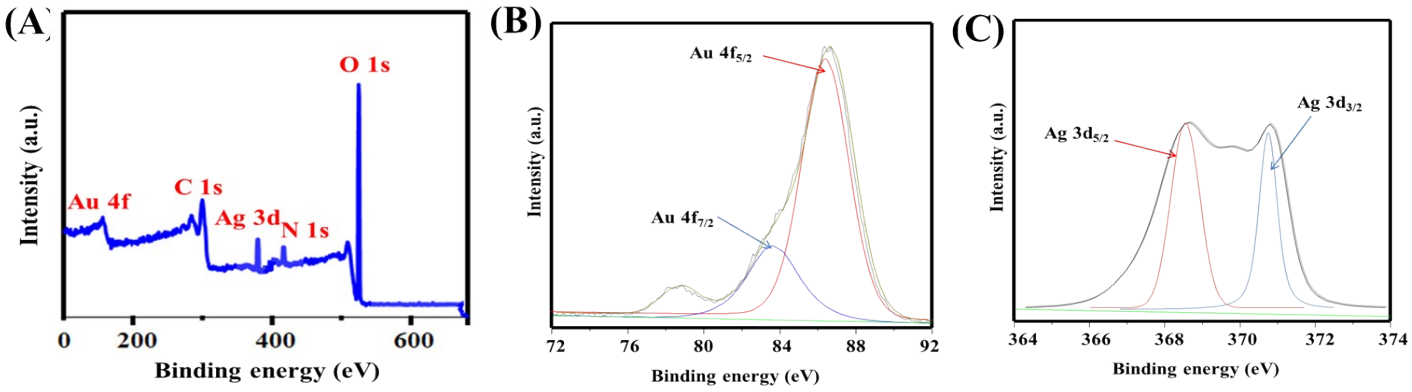


**Fig.S2** (A) XPS of LYS@Ag/Au NCs; (B) High resolution XPS of Au 4f; (C) High resolution of Ag 3d.

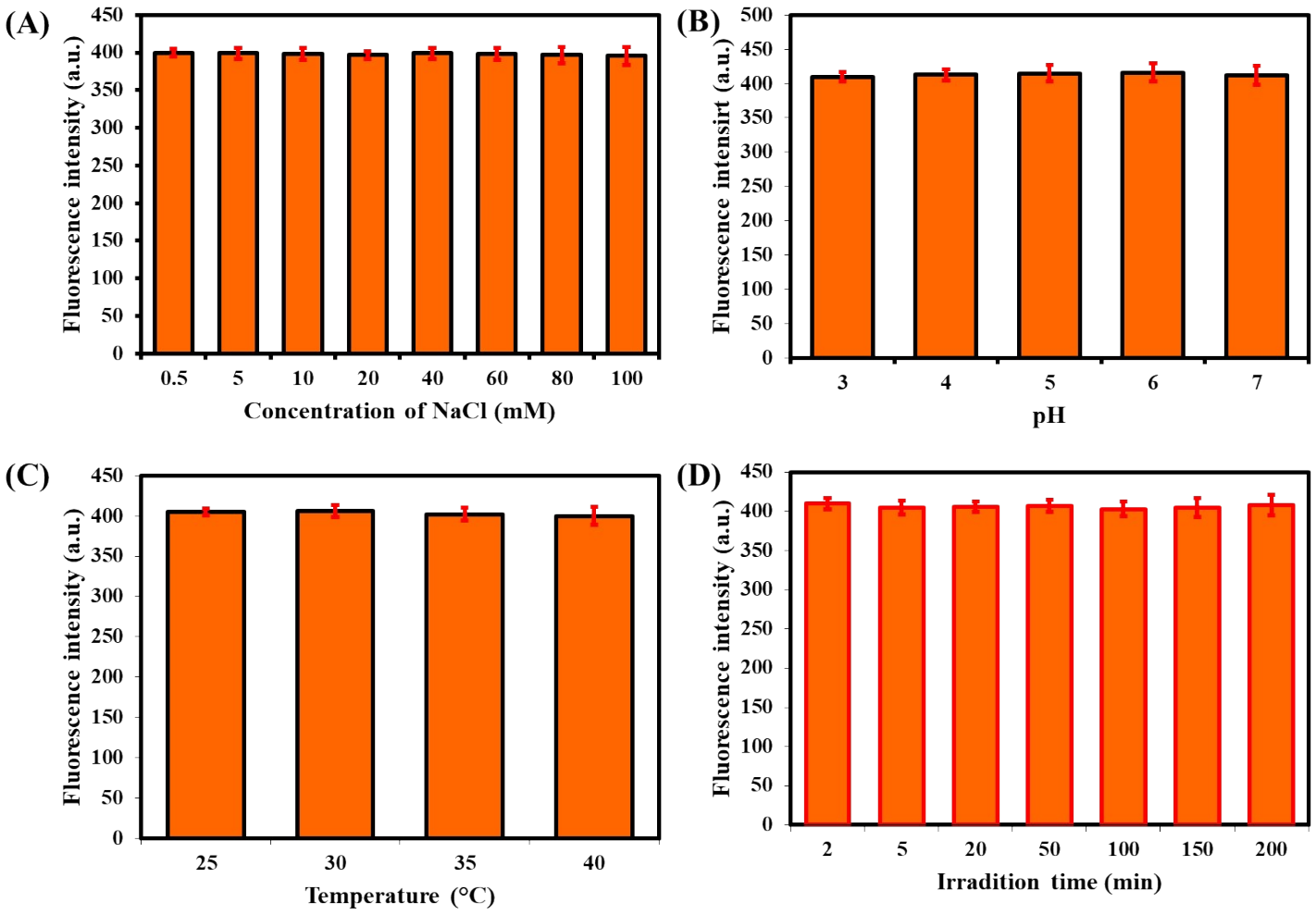


**Fig.S3** The effect of NaCl concentration (A), pH (B), temperature (C), and irradiation time (D) on the stability of LYS@Ag/Au NCs.

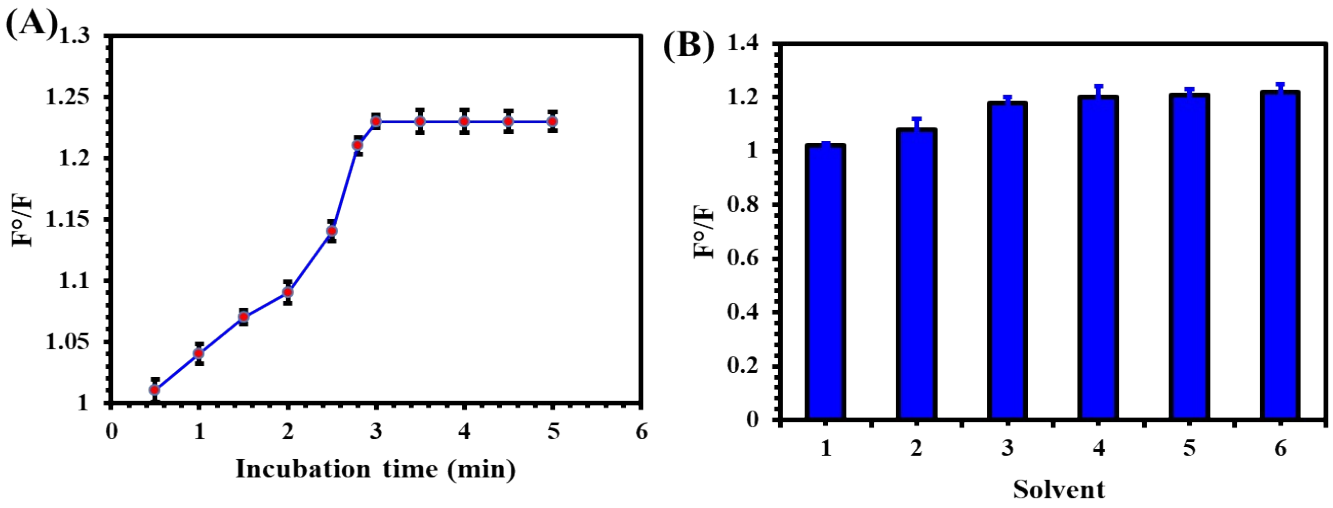


**Fig.S4** (A) The influence of reaction time on the fluorescence emission of LYS@Ag/Au NCs in the presence of 300 µM histamine. (B) Effect of diluting solvent on the fluorescence emission of LYS@Ag/Au NCs in the presence of 30 µM histamine using various 0.1 M diluting solvents: (1) HCl, (2) NaOH, (3) Citrate buffer saline, (4) Acetate buffer saline, (5) Phosphate buffer saline, and (6) Water.

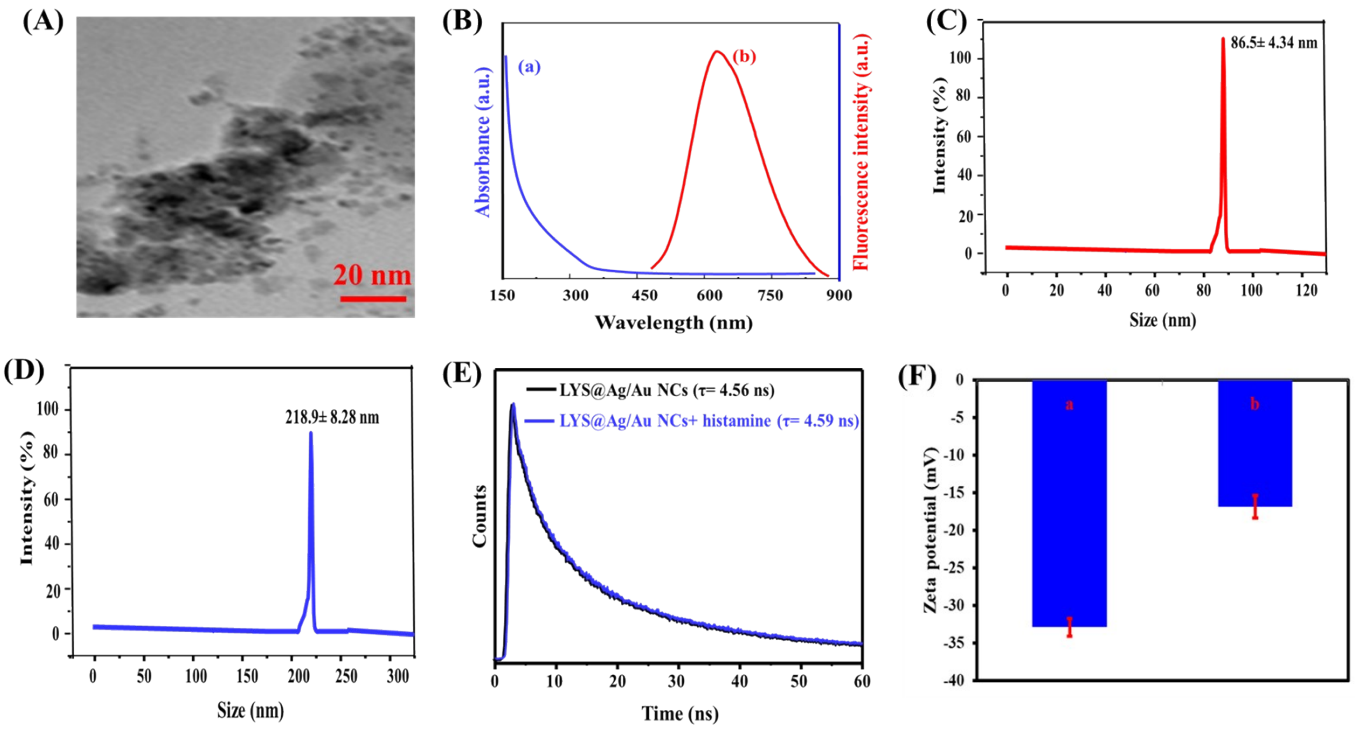


**Fig.S5** (A) TEM image of LYS@Ag/Au NCs after addition of 60 μM histamine; (B) UV/Vis absorption spectrum of 60 μM histamine (a) and fluorescence emission spectrum of LYS@Ag/Au NCs (b); (C) DLS of LYS@Ag/Au NCs; (D) DLS of LYS@Ag/Au NCs after addition of 60 μM histamine; (E) Fluorescence life times of LYS@Ag/Au NCs in absence and presence of 60 μM histamine; (F) Zeta potentials of LYS@Ag/Au NCs and LYS@Ag/Au NCs/ 60 μM histamine.

**Table S1** Recovery of the determination of histamine in serum samples (n=5).

| Sample | Added (μM) | LYS@Ag/Au NCs | | |
|---|---|---|---|---|
| | | Found (μM) | Recovery % | RSD |
| A | 0.0 | ND | ----- | ----- |
| | 0.5 | 0.493 | 98.6 | 3.36 |
| | 3.0 | 3.022 | 100.7 | 3.28 |
| | 5.0 | 4.850 | 97.0 | 2.45 |
| B | 0.0 | ND | ----- | ----- |
| | 0.5 | 0.504 | 100.8 | 3.55 |
| | 3.0 | 2.981 | 99.4 | 2.89 |
| | 5.0 | 5.042 | 100.8 | 3.28 |
| C | 0.0 | ND | ----- | ----- |
| | 0.5 | 0.492 | 98.4 | 3.44 |
| | 3.0 | 3.07 | 102.3 | 3.56 |
| | 5.0 | 4.974 | 99.5 | 2.37 |

**Table S2** Recovery of the determination of histamine in canned tuna samples (n=5).

| Sample | Added (mg $Kg^{-1}$) | LYS@Ag/Au NCs | | |
|---|---|---|---|---|
| | | Found (mg $Kg^{-1}$) | Recovery % | RSD |
| A | 0.0 | 16.08 | ----- | ----- |
| | 0.5 | 16.25 | 100.7 | 3.54 |
| | 3.0 | 19.56 | 102.5 | 2.87 |
| | 5.0 | 20.87 | 99.0 | 4.23 |
| B | 0.0 | 15.55 | ----- | ----- |
| | 0.5 | 15.23 | 97.6 | 3.98 |
| | 3.0 | 18.56 | 100.1 | 4.09 |
| | 5.0 | 21.32 | 103.7 | 3.58 |
| C | 0.0 | 17.35 | ----- | ----- |
| | 0.5 | 17.08 | 98.2 | 3.76 |
| | 3.0 | 20.90 | 102.7 | 3.24 |
| | 5.0 | 23.34 | 104.4 | 4.06 |