Supplementary information

Colorimetric visualization of histamine secreted by basophils based on DSP-functionalized gold nanoparticles

Xiaojing Ling[a#], Yongxin Liu[b#], Danxuan Zhu[c#], Weizhen An[b], Jiaying Geng[b], Lin Li[b], Changmin Yu[b], Ji-Fu Wei[a,c*]*

[a] Department of Pharmacy, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China. E-mail: weijifu@njmu.edu.cn

[b] Key Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM), Nanjing Tech University (NanjingTech), 30 South Puzhu Road, Nanjing 211800, P. R. China. E-mail: iamcmyu@njtech.edu.cn

[c] Women & Children Central Laboratory, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210036, China.

*** Corresponding authors:**

Prof. Ji-Fu Wei, Department of Pharmacy, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing 210009, China.
E-mail: weijifu@njmu.edu.cn

Prof. Changmin Yu, Key Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM), Nanjing Tech University (NanjingTech), 30 South Puzhu Road, Nanjing 211800, P. R. China.
E-mail: iamcmyu@njtech.edu.cn

[#] Xiaojing Ling, Yongxin Liu and Danxuan Zhu contributed equally to this paper.

1. Instruments and reagents

1.1 Instruments

The centrifuge used for this study was a 5424R refrigerated centrifuge from Eppendorf (China) Co., Ltd. Zeta potential was measured on a Nanoplus particle size analyzer (Shanghai McMuritik Instrument Co., Ltd.). A UV-vis-1780 microplate reader (Shimadzu, Japan) was used to detect the adsorption values of AuNPs and DSP-AuNPs. The morphology of AuNPs and DSP-AuNPs was characterized by TEM (JEM2100 PLUS, Japan). The infrared spectra of AuNPs, DSP-AuNPs and DSP-AuNPs in the presence of 2 μM histamine were measured on a Tensor II FTIR spectrometer (Bruker, Germany). Raman spectra of DSP-AuNPs were detected by iHR 550 laser confocal Raman microspectroscopy (Horiba, Japan). The DLS of AuNPs, DSP-AuNPs and DSP-AuNPs in the presence of 1 μM, 4 μM and 8 μM histamine was detected by a Malvern Zetasizer Nano analyzer. Basophil expressions of CCR3 and CD63 were evaluated using FACS Canto Plus and analyzed by the build-in Diva software (BD Bioscience, California, USA).

1.2 Regents

Gold trichloride solution and trisodium citrate were purchased from Aladdin. All chemical solvents, amino acids and inorganic salts were purchased from Shanghai Titan Technology Co., Ltd. Dimethyl sulfoxide (DMSO) was purchased from Saiguo Bio. Purified water was purchased from Wahaha. All reagents were used directly without any purification. Milli-Q-grade water was used for all experiments (Ω = 18.25).

1.3 Synthesis of AuNPs

The synthesis of AuNPs was performed according to the previous Turkevich method [1]. First, all the glass instruments used for the experiment were washed with aqua regia (HCl: HNO_3 = 3:1) and further washed with DI water. Gold trichloride solution (0.01%, 25 μL) with DI water (100 mL) was added to a 250 mL round bottom flask, which was placed on a magnetic stirrer with rapid stirring (1000 rpm). When the temperature of the solution rose to 160 - 180°C, sodium citrate solution (1%, 3.64 mL) was quickly added. The color of the bowling solution rapidly changed from pale yellow to wine red, and it was continuously stirred for 25 min. The heating was stopped, but the stirring continued for 20 min. When cooled to room temperature, the solution was centrifuged (13000 rpm, 15 min, 4°C). Synthesized AuNPs were resuspended in DI water and stored in a clean glass bottle at 4°C. The absorption peak of synthesized AuNPs was detected by a UV-vis microplate reader. The morphology and diameter of the synthesized AuNPs were characterized by TEM.

1.4 Design and preparation of the AuNP probe for histamine

An imidazole ring and a side-chain aliphatic amino group linked by a two-carbon-atom chain constitute histamine molecules [2]. The imidazole ring can stably connect to the surface of AuNPs, and DSP molecules react to the side-chain aliphatic amino group, narrowing the distance of AuNPs and finally causing their aggregation [3, 4].

First, 50 mM DSP dissolved in DMSO was prepared and stored at -20°C. Then, the 500 μM DSP solution was prepared via 50 μL of 50 mM DSP added to 4.95 mL of DI water. Then, 70 μL of 500 μM DSP with 5 mL of 10 nM freshly prepared citrate-stabilized AuNPs was added to a 25 mL round bottom flask, which

was placed on a magnetic stirrer with soft stirring (300 rpm) at room temperature for 30 min. Subsequently, DSP-AuNPs were washed. After centrifugation, DSP-AuNPs were resuspended in 0.1 M HEPES buffer (pH 7.0), which was stored at 4°C in a clean glass bottle. The absorbance of DSP-AuNPs was detected by a UV-vis microplate reader. The Raman spectra analyzer and the zeta potential analyzer were used to characterize whether DSP was modified to the surface of AuNPs. Additionally, the morphology and diameter of DSP-AuNPs were also characterized by TEM.

1.5 FTIR and Raman spectra

Then, 20 mL of AuNPs, DSP-AuNPs and DSP-AuNPs in the presence of 2 μM histamine were precooled at -80°C and freeze-dried overnight. Lyophilized samples were detected by a FTIR spectrometer. Then, the Raman spectra of DSP-AuNPs were detected by iHR 550 laser confocal Raman microspectroscopy.

1.6 The DLS analysis

The samples including AuNPs, DSP-AuNPs and DSP-AuNPs in the presence of 1 μM, 4 μM and 8 μM were prepared, which were resuspended in DI water. The DLS of these samples was detected by a Malvern Zetasizer Nano analyzer.

1.7 The optimized conditions of the DSP-AuNP assay for histamine

The 0.1 M HEPES buffer with different pH values (pH = 4.0, 5.0, 6.0, 6.5, 7.0, 7.4, 7.6, 8.0 and 8.5) was prepared, and 140 μL of 500 μM DSP with 10 mL of 10 nM AuNPs was mixed and stirred for 30 min at room temperature. After washing, DSP-AuNPs were resuspended in 0.1 M HEPES buffer with different pH values (pH = 4.0, 5.0, 6.0, 6.5, 7.0, 7.4, 7.6, 8.0 and 8.5). 90 μL of DSP-AuNPs in 0.1 M HEPES buffer with different pH values with the addition of 10 μL of DI water or 10 μL of histamine samples at a final concentration of 1 μM were mixed. The ratio of absorption values of DSP-AuNPs with DI water or histamine samples at a final concentration of 1 μM at 650 nm and 520 nm was used to assess the stability and responsiveness of the DSP-AuNP probe for histamine.

Subsequently, the concentration of DSP modified to the surface of AuNPs was optimized. AuNPs (10 nM) with the addition of DSP at different concentrations of 5, 7, 10 and 12 μM were softly stirred at room temperature for 30 min to synthesize DSP-AuNPs. After washing, the modified AuNPs were resuspended in 0.1 M HEPES buffer (pH 7.0). 90 μL of DSP-AuNPs in 0.1 M HEPES buffer (pH 7.0) was mixed with 10 μL of DI water or 10 μL of histamine samples at a final concentration of 1 μM. The optimized concentration of DSP modified to the surface of AuNPs was confirmed by the stability and responsiveness of the DSP-AuNP probe for histamine, which depended on the ratio of absorption values of DSP-AuNPs with DI water or histamine samples at a final concentration of 1 μM at 650 nm and 520 nm. Similarly, the optimized concentration of DSP-AuNPs was also confirmed.

Finally, the optimized temperature of the DSP-AuNP assay for histamine was confirmed. 10 μL of histamine samples at a final concentration of 2 μM was added to 90 μL of DSP-AuNPs, which were incubated at 4, 25, 37 and 44°C for 5 min. Their absorbance was detected by a UV-vis microplate reader ranging from 400 to 800 nm.

1.8 Kinetics analysis of DSP-AuNP assay for histamine

The kinetics of the DSP-AuNP assay for histamine at a final concentration of 2 μM were analyzed by a UV-vis microplate reader. In detail, 90 μL of DSP-AuNPs mixed with 10 μL of 20 μM histamine samples was added to the microplate, and the absorbance of the DSP-AuNP assay for histamine at a final concentration of 2 μM was detected by a UV-vis microplate reader for approximately 15 min with a 30 s interval. The data of kinetic analysis were presented as mean (the ratio of A_{650}/A_{520} values)/standard derivation (the ratio of A_{650}/A_{520} values) (n = 3).

1.9 Selectivity and sensitivity for *in vitro* histamine detection

The selectivity of the DSP-AuNP probe for histamine was analyzed under the optimized conditions. Briefly, 24 interferents including various inorganic salts and amino acids were first resolved in DI water. Additionally, the biological amine (putrescine, cadaverine) and protein (BSA) samples in DI water were also prepared. 90 μL of DSP-AuNPs with 10 μL of interferent samples at a final concentration of 10 μM were mixed. Subsequently, the absorbance of DSP-AuNPs with various interferents was detected by a UV-vis microplate reader. The ratio of absorption values of DSP-AuNPs with different interferents at final concentrations of 10 μM at 650 nm and 520 nm was used to assess the selectivity of the DSP-AuNP probe for histamine. Similarly, 90 μL of DSP-AuNPs with the ordered addition of 5 μL of 200 μM histamine samples and 5 μL of 200 μM individual interferent, which was used to further assess the tolerability of the DSP-AuNP probe for histamine in the presence of individual interferents.

Histamine samples at different concentrations ranging from 0 to 100 μM were prepared in DI water. 10 μL of histamine samples was added to 90 μL of freshly prepared DSP-AuNPs, which was incubated for 5 min at room temperature. Subsequently, the absorbance of DSP-AuNPs with histamine samples at different concentrations was detected by a UV-vis microplate reader ranging from 400 to 800 nm.

1.10 The stability of the DSP-AuNP assay for histamine

Freshly prepared DSP-AuNPs with the addition of histamine were stored at 4°C. Then, the absorbance of DSP-AuNPs in the presence of histamine was detected on days 0, 15, 30 and 45, and their absorbance was compared with that of DSP-AuNPs stored for day 0.

1.11 MTT

BMMCs were cultivated in the presence of IL-3 and stem cell factor. Then, 100 μL of mature BMMCs (approximately 5000/well) with the addition of DSP-AuNPs at different concentrations of 0, 0.5, 1, 2, 3 and 4 nM was added to a 96-well plate, which was incubated in a cell incubator (37°C, 5% CO_2) for 10 h. Then, 10 μL of MTT was added to the mix of mast cells and DSP-AuNPs, which was incubated in a cell incubator (37°C, 5% CO_2) for 4 h. After centrifugation, mast cells were suspended in DMSO. Finally, their absorption values were read by a UV-vis microplate reader at 490 nm. The cell activity was presented as the DSP-AuNP group / (the positive control - blank).

1.12 Recovery rate

Histamine samples at different concentrations ranging from 0 to 100 μM were prepared in DI water. Then, 5 μL of 8 μM histamine samples with 5 μL of 100 μM histamine samples, 5 μL of 8 μM histamine samples with 5 μL of 200 μM histamine samples and 5 μL of 4 μM histamine samples with 5 μL of 400 μM histamine

samples were added to 90 μL of DSP-AuNPs. Their absorbance was detected by a UV-vis microplate reader ranging from 400 to 800 nm at room temperature, and their respective ratio of absorption values at 650 nm and 520 nm was calculated. Subsequently, their respective concentrations were calculated by the standard curve acquired in the study. The recovery rate was presented as the found concentration/known concentration × 100% (n = 3).

1.13 The DSP-AuNP assay for histamine secreted by activated basophils

200 μL of fresh peripheral blood from the volunteer with 4 mL of 1 × red blood lysis buffer was mixed and further incubated for 12 min at room temperature to break red blood cells. The pelleted cells were washed with PBS washing buffer (PBS-0.1% fetal bovine serum) and Tris-buffer (25 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.6) to remove broken red blood cells. Subsequently, peripheral white blood cells were resuspended in 100 μL of Tris-A buffer (Tris buffer containing 0.6 mM Ca^{2+}, 1 mM Mg^{2+}, pH 7.6). Then, isolated leukocytes with the addition of fMLP, as an IgE-independent activator specific for basophils, at a final concentration of 10 μg/mL were incubated for 20 min in a cell incubator to activate basophils. The supernatant of the above cell suspension was obtained after centrifugation (1000 rpm, 20°C, 5 min). Then, 20 μL of the supernatant (1:2 diluted in Tris-A buffer) was added to 80 μL of DSP-AuNPs to determine the histamine secreted by activated basophils.

2. Results and tables

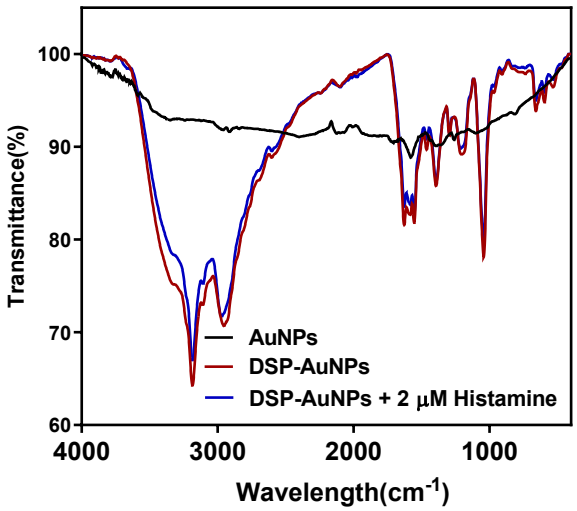


Figure S1. FITR spectra of AuNPs, DSP-AuNPs and DSP-AuNPs in the presence of histamine.

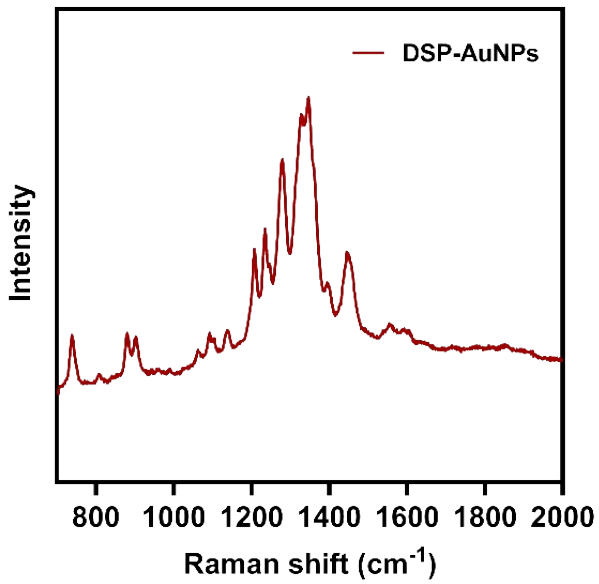


Figure S2. Raman spectra of DSP-AuNPs.

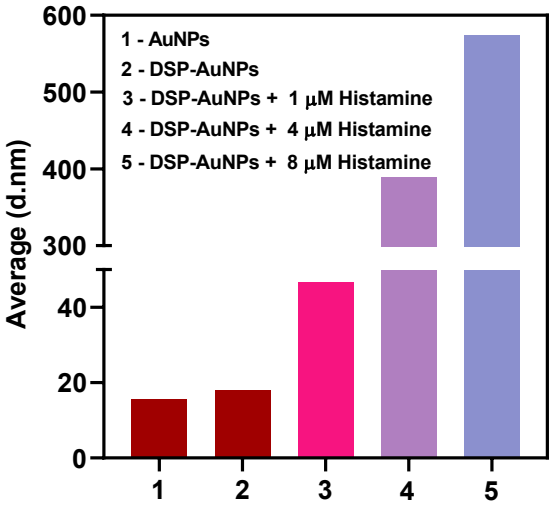
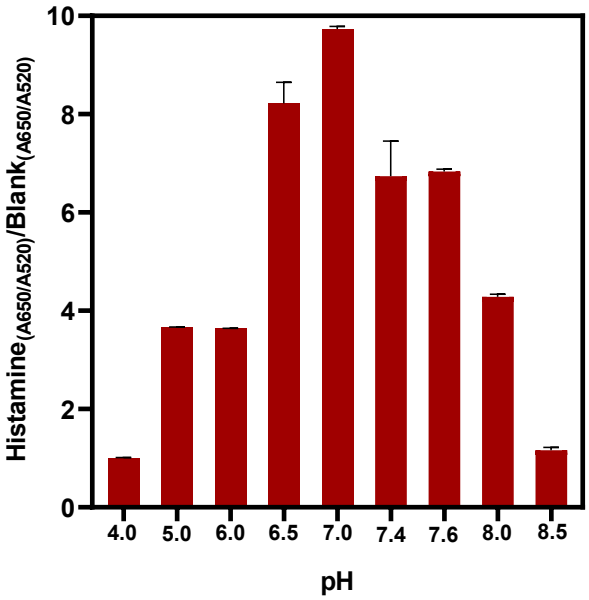


Figure S3. The DLS analysis of AuNPs, DSP-AuNPs and DSP-AuNPs in the presence of 1 µM, 4 µM and 8 µM histamine.



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172 **Figure S4. The optimized pH.** DSP-AuNPs in 0.1 M HEPES buffer (pH 7.0) had the best response to

173 histamine at a final concentration of 1 μM.

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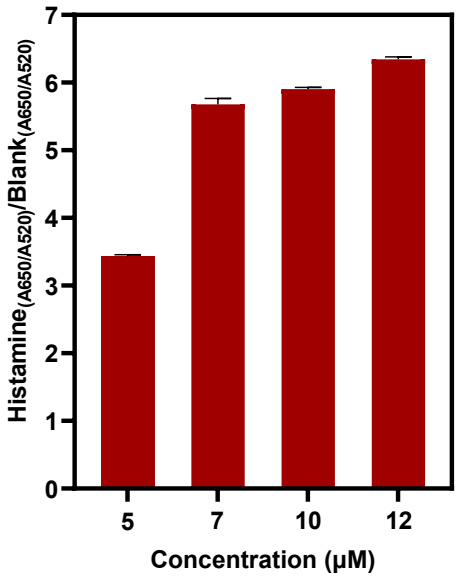


Figure S5. The optimized concentration of DSP. AuNPs (10 nM) with the addition of DSP at different concentrations of 5, 7, 10 and 12 μM were softly stirred for 30 min at room temperature to synthesize DSP-AuNPs. In the presence of 12 μM DSP, DSP-AuNPs had a better response to histamine at a final concentration of 1 μM. However, DSP-AuNPs caused a great loss during washing when the concentration of DSP was over 7 μM.

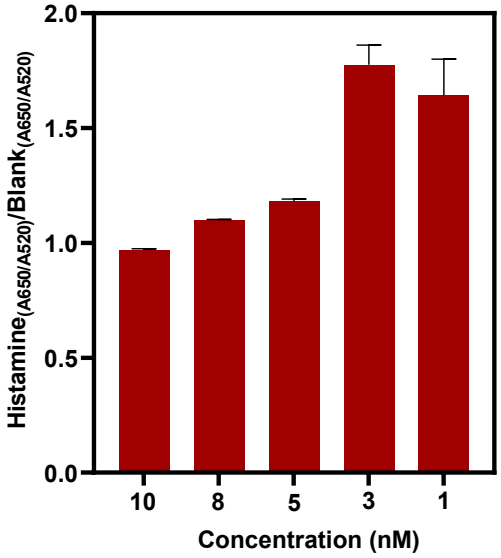
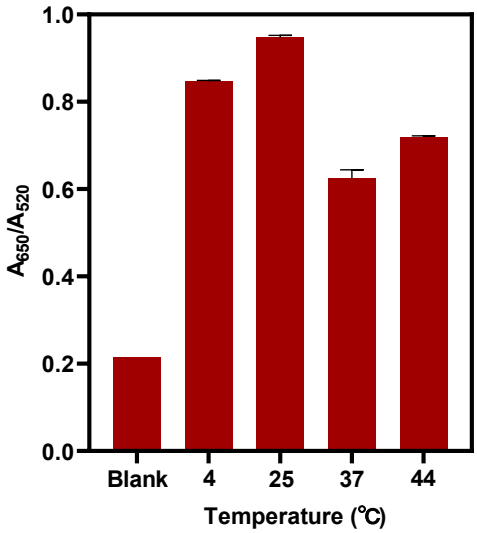


Figure S6. The optimized concentration of DSP-AuNPs. With the addition of histamine at a final concentration of 1 μM, the concentration of DSP-AuNPs (3 nM) was optimized.



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185 **Figure S7. The optimized temperature of the DSP-AuNP assay for histamine.**

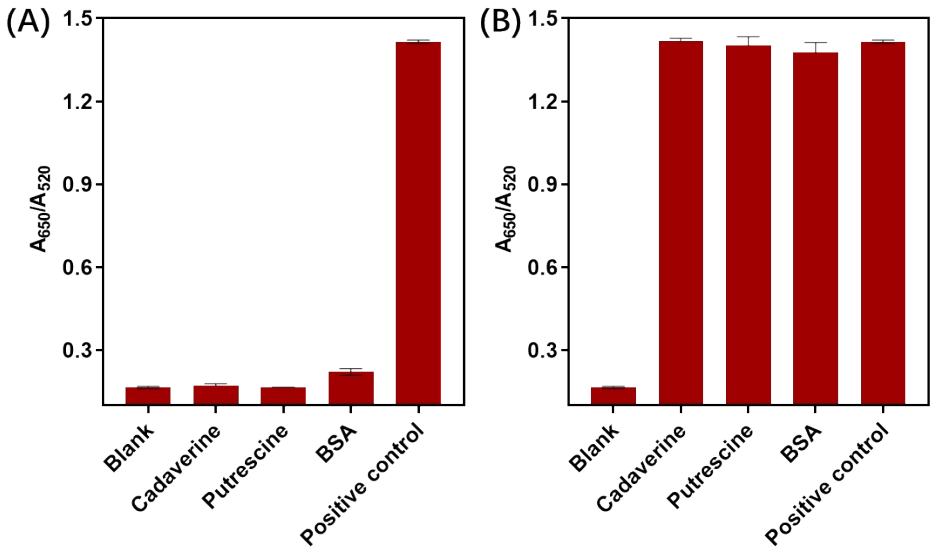

Figure S8. The responsiveness of DSP-AuNPs to cadaverine, putrescine and BSA. (A) The selectivity of the DSP-AuNP assay for histamine. (B) Representation of A_{650}/A_{520} for a mix of histamine (10 μM) with cadaverine/putrescine/BSA.

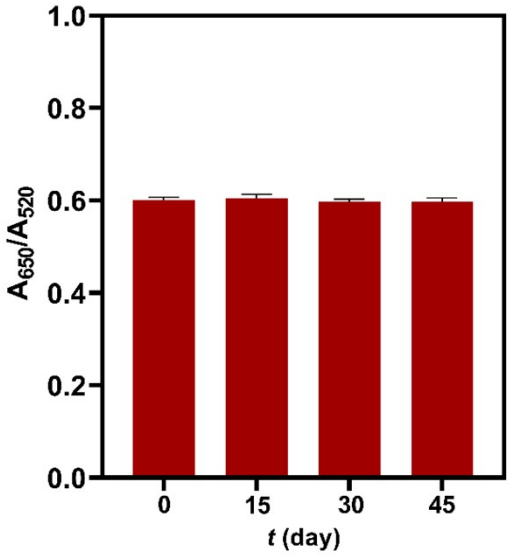

Figure S9. The stability of the DSP-AuNP assay for histamine.

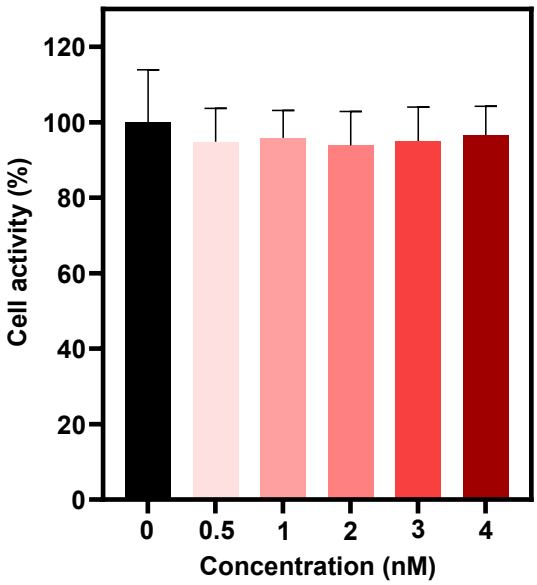

Figure S10. MTT. BMMCs with the addition of DSP-AuNPs at different concentrations of 0, 0.5, 1, 2, 3 and 4 nM were incubated for 10 h.

Table S1. Comparison with other colorimetric methods for detecting histamine based on AuNPs

Analyte	Linear range (μM)	LOD (μM)	Sample	References
Histamine and histidine	0.001-10.0	0.87×10^{-3}	Salmon Muscle	3
Histamine	2.0-16.0	0.6	Chicken	5
Histamine	0.1-2.1	0.038	Frozen salmon	6
Histamine	0.2-0.4	0.2	Wine	7
Histamine	0-449.8	0.315	Meat, fish and crustaceans	8
Histamine	6.08-35.68	0.008	Fish samples	9
Histamine	0.8-2.5	0.014	Human blood basophils	This work

Table S2. Recovery rate

Known concentration (μM)	Found concentration (μM)	Recovery rate (%)
0.9	0.843418074	93.71%
1.4	1.224311689	87.45%
2.2	2.223699005	101.08%

3. References

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