Supporting Information

Sensitive and multicolor detection of nitrite based on iodide-mediated etching of gold nanostars

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Experimental Section

Reagents and instruments

Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), sodium citrate dihydrate, hydroxylamine hydrochloride, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethane-sulfonic acid (HEPES), potassium iodide (KI), sodium nitrite ($NaNO_2$), hydrochloric acid, and sodium hydroxide were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cabbage and sausages were purchased from the local supermarket (Xiamen, China). All aqueous solutions were prepared using deionized water obtained from the Millipore water purification system (Sartorius, Germany) with a resistivity of 18.2 MΩ cm. The UV–Vis absorption spectra were measured using a SpectraMax Plus384 microplate reader (Molecular Devices, America). Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images were recorded using a Tecnai G2 F30 transmission electron microscopy (FEI, America). The crystal structures of Au NSs structures were determined by X-ray powder diffraction (XRD, Bruker D8 Advance).

Synthesis of Au NSs

The Au NSs was prepared by a seed-mediated method following the reported methodologies.[1] Gold nanoparticles (Au NPs) with a size of ~ 13 nm were used as seeds.[2] 375 μL of the Au NPs solution was added to 375 μL of 40 mM hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) solution, followed by the addition of 10 mL of HEPES (100 mM) and 10 mL of ultrapure water. The solution was mixed under stirring for 5 min. Then, $HAuCl_4$ solution (10 mL, 1mM) was slowly added to the above solution and further reacted for 15 min under stirring at room temperature. After that, the solution was centrifuged (6000 rpm, 15 min) at 4 °C. Finally, ultrapure water was added to obtain Au NSs solution (~120 μg mL^{-1} Au).

Nitrite sensing

Twenty μL KI (15 μM), 50 μL $NaNO_2$ standard solution (0, 2, 5, 10, 20, 50, 100, 200, 300, 500, 1000 μM) and 10 μL HCl (0.8 M) were successively added to microplates. After the mixture was evenly mixed, it was incubated at room temperature for 30 min. Then, 40 μL NaOH (0.2 M) was added to the above solutions. After the pH of the solution was adjusted to neutral, 80 μL of 10 mM HAc-NaAc (pH 5) buffer was added to adjust the pH of the reaction system to pH 5. Eventually, 50 μL Au NSs were added. The mixture was incubated for 30 min and the UV–Vis

absorption spectra were recorded.

Evaluation of the selectivity toward nitrite

To further investigate the specificity of this assay, 50 μL NaNO$_2$ (300 μM) and possibly interfering ions CO$_3^{2-}$, SO$_4^{2-}$, Ac$^-$, Cl$^-$, HPO$_4^{2-}$, Br$^-$ and NO$_3^-$ (1000 μM) were respectively added to 96-well plates. Then, 20 μL KI (15 μM) and 10 μL HCl (0.8 M) solution were added in sequence. The mixture was mixed evenly and incubated for 30 min at room temperature. Then, 40 μL NaOH (0.2 M) was added to adjust the pH of the solution to neutral. Subsequently, 80 μL HAc-NaAc (pH 5, 10 mM) was added. After the mixture was evenly mixed, 50 μL Au NSs were added. Then, incubation was continued for an additional 30 min. The color changes of the solution were observed, and the UV–Vis absorption spectra were recorded. All samples were tested three times to evaluate the effects of these potentially interfering ions.

Assay of nitrite in food samples

To verify the practicability of the proposed method, the nitrite content of cabbage and sausage samples was quantitatively analyzed. The samples were pretreated according to the previous reports with minor modifications.[3] Briefly, 5 g of homogenized sample was weighed and placed in a 150 mL Erlenmeyer flask with 80 mL of ultrapure water and 1 mL of KOH solution. The sample was ultrasonicated for 30 min and then heated in a 75 °C water bath for 5 min, followed by removal of the sample and cooling to room temperature. Then, the sample was quantitatively transferred to a 100 mL volumetric flask, and diluted to the mark with water and shaken well. The turbid solution was centrifuged at 8000 rpm for 10 min and the resulting supernatant was collected. Finally, 2 g of activated carbon was added to the collected supernatant and mixed for 5 min. After centrifugation again, the supernatant was filtered through a 0.22 μm pore size filter membrane, and the filtrate was collected for further detection. All samples were analyzed three times to assess the repeatability of our assay. In addition, the content of nitrite in the samples was detected by spectrophotometry according to the China National Food Safety Standard (GB 5009.33–2016) for comparison to evaluate the accuracy of our method.[4]
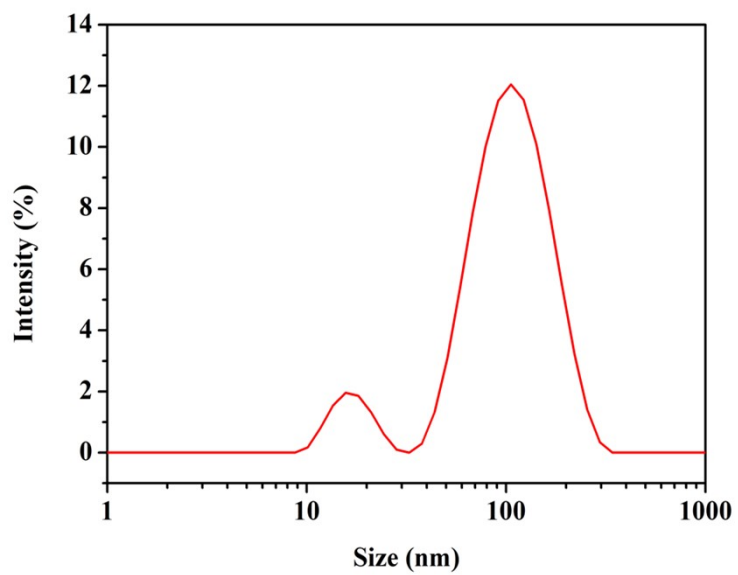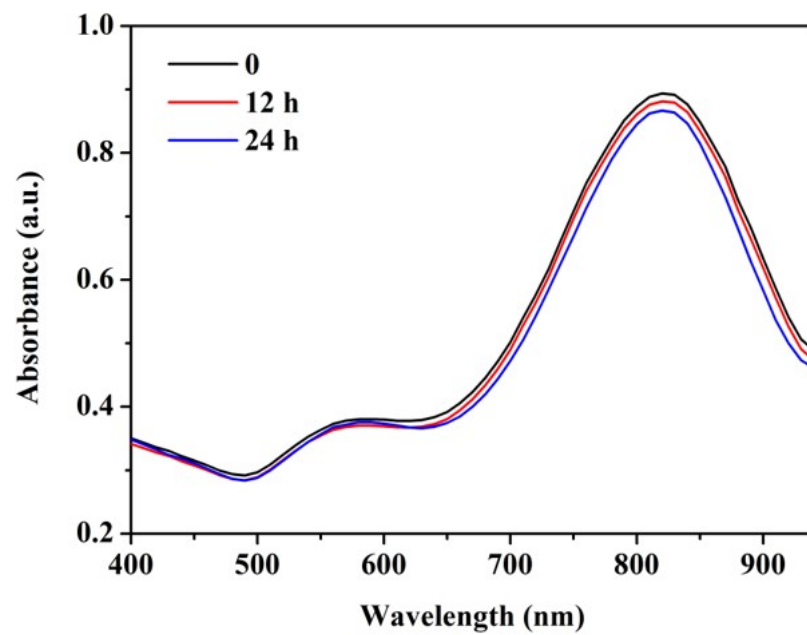
**Figure S1**. Dynamic Light Scattering (DLS) measurement of Au NSs.
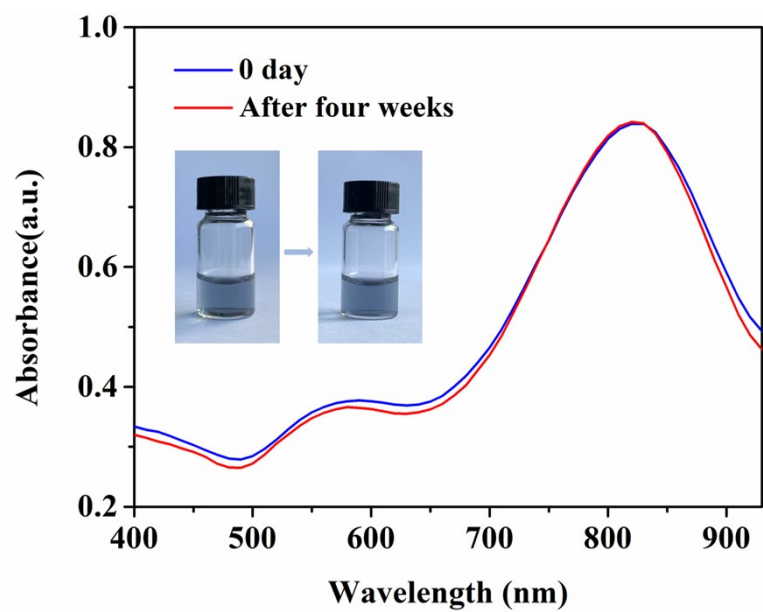
Figure S2. UV–Vis absorption spectra and corresponding photographs of Au NSs after storage at 4 °C for four weeks.
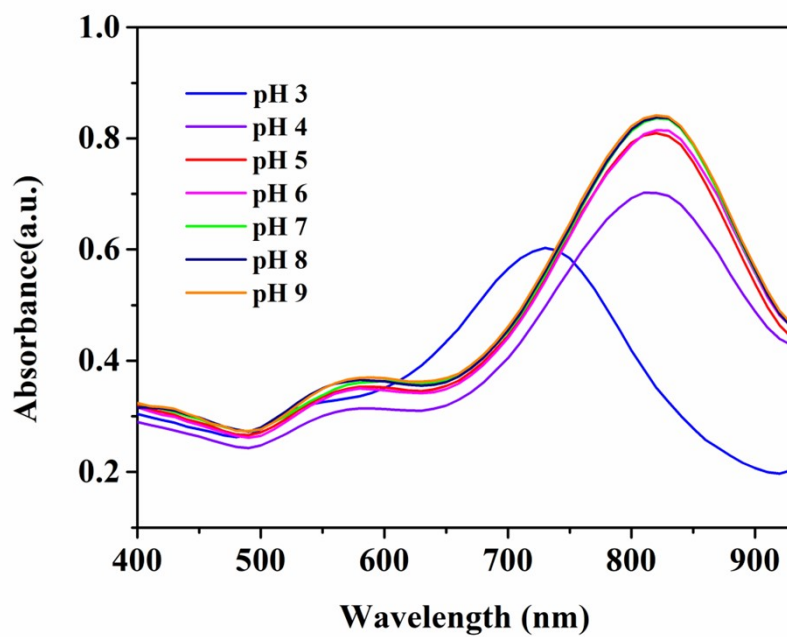
Figure S3. UV–Vis absorption spectra of Au NSs in solutions with different pH values (pH 3, 4, 5, 6, 7, 8, 9) after 60 min.
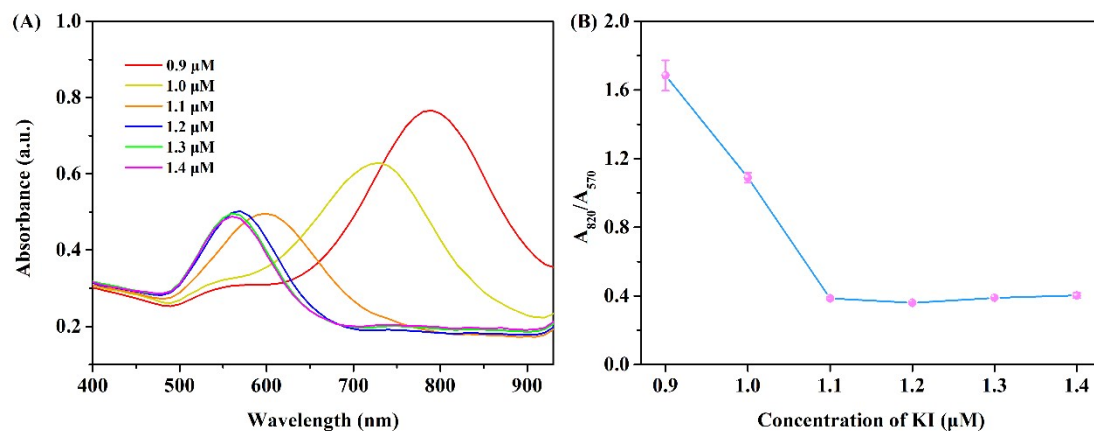
Figure S4. Uv–Vis absorption spectra of Au NSs in NO_2^- (1 mM) solution at room temperature for 0 (black line), 12 (red line) and 24 h (blue line).
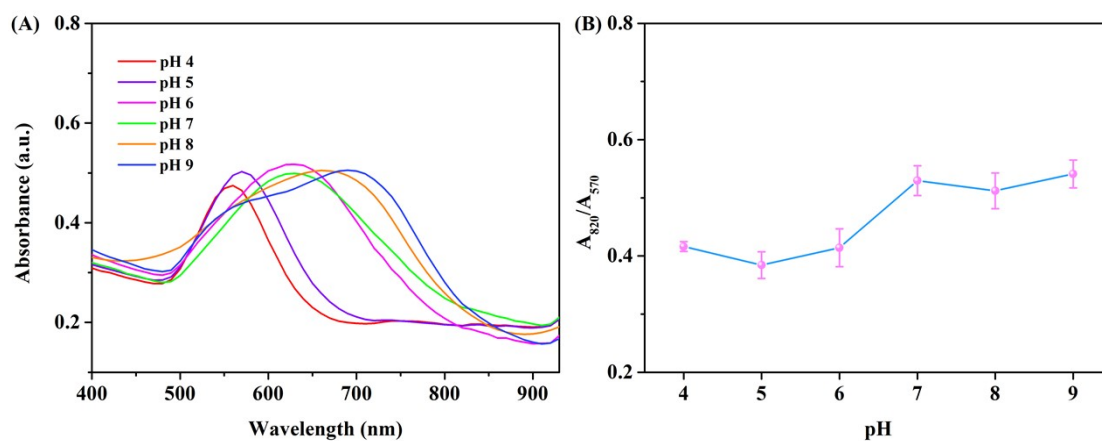
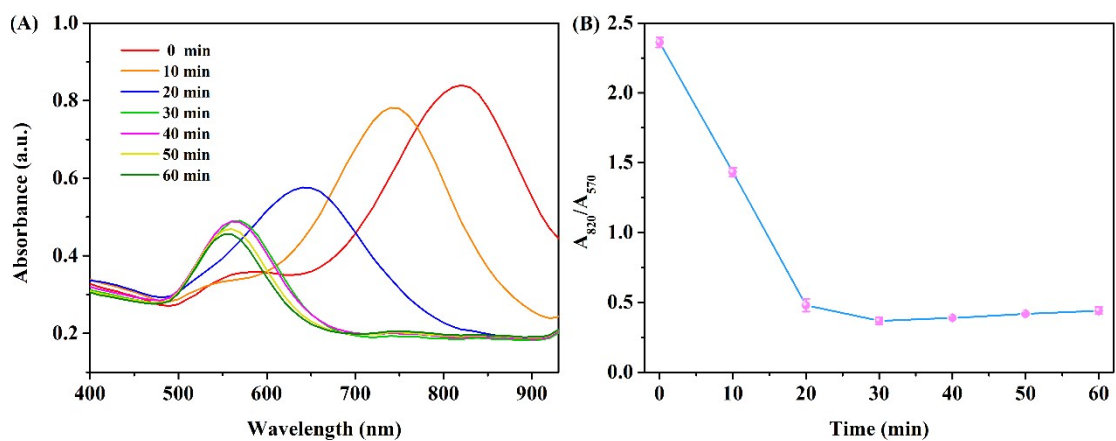**Figure S5.** (A) UV–Vis absorption spectra of Au NSs etched by 1.2 μM iodide at different pHs. (B) The absorbance ratio (A_{820}/A_{570}) plotted as a function of pH values.
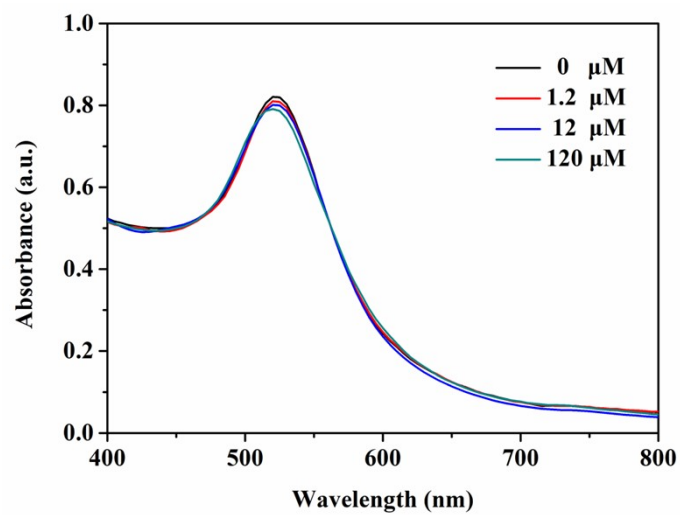
Figure S6. (A) UV–Vis absorption spectra of Au NSs etched with varying iodide concentrations.

(B) The absorbance ratio (A_{820}/A_{570}) plotted as a function of iodide concentration.

Figure S7. (A) UV–Vis absorption spectra of Au NSs etched by 1.2 μM iodide with varying reaction times. (B) The absorbance ratio (A_{820}/A_{570}) plotted as a function of reaction times.

Figure S8. UV–Vis absorption spectra of Au NPs incubated with different iodide concentrations ranging from 0 to 120 μM.
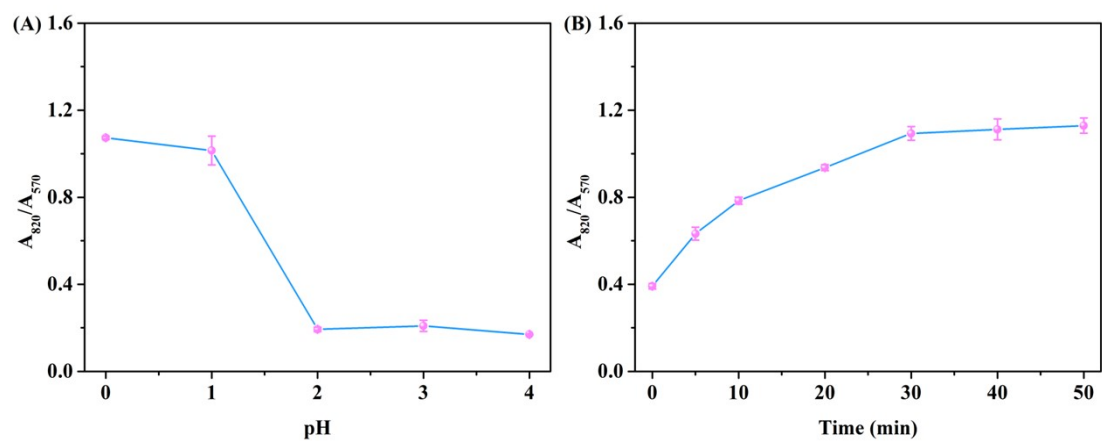
Figure S9. Optimization of redox reaction conditions between nitrite and iodide. (A) pH optimization of iodide oxidation by nitrite. (B) Reaction time optimization of iodide oxidation.

Table S1 Performance comparison of colorimetric sensors for the detection of nitrite.

Material	Response Time (min)	LOD (μM)	Detection range (μM)	Reference
Au nanorods	20	1.3	8-100	5
Au nanoparticles	120	4.5	10-1000	6
His@AuNCs/RGO	5	2	10-500	7
AuNP-CeO$_2$ NP@GO	1.5	4.6	100-5000	8
Ag nanoparticles	35	0.149	0.35-22	9
Hollow MnFeO	55	0.2	3.3-133.3	10
Au nanostars	60	0.4	2-300	This work

Table S2 Analytical results of nitrite in actual samples by iodide-mediated etching of Au NSs and the standard method.

Samples	Spiked levels (μM)	The developed method			The standard method			P[c]
		Found±SD[a] (μM)	Recovery (%)	RSD[b] (%)	Found±SD (μM)	Recovery (%)	RSD (%)	
Cabbage	0	N.D[d]	-	-	N.D	-	-	
	5	4.66±0.07	93.2	1.5	5.03±0.15	100.6	3.0	0.26
	10	9.89±0.33	98.9	3.3	9.85±0.31	98.5	3.1	
Sausage I	0	4.26±0.24	-	5.6	4.14±0.09	-	2.2	
	5	9.26±0.43	100.0	4.6	9.41±0.42	105.4	4.5	0.77
	10	14.69±0.58	104.3	3.9	14.48±0.53	103.4	3.7	
Sausage II	0	3.68±0.14	-	3.8	3.49±0.11	-	3.2	
	5	8.27±0.41	91.8	5.0	8.63±0.31	102.8	3.6	0.68
	10	13.77±0.45	100.9	3.3	13.39±0.43	99.0	3.2	

[a] SD: standard deviation, n=3.

[b] RSD: relative standard deviation.

[c] P: P > 0.05 indicating no significant difference.

[d] N.D: not detected.

References

1 Y. Xianyu, S. Su, J. Hu and T. Yu, *Biosens. Bioelectron*., 2021, **190**, 113430.

2 S. Guo, L. Wang, S. Dong and E. Wang, *J. Phys. Chem. C*, 2008, **112**, 13510-13515.

3 M. Yu, H. Zhang, Y. Liu, Y. Zhang, M. Shang, L. Wang, Y. Zhuang and X. Lv, *Food Chem*., 2022, **374**, 131768.

4 GB 5009.33–2016. National Standards of the People's Republic of China, 2016.

5 M. Sepahvand, F. Ghasemi and H. M. S. Hosseini, *Anal. Methods*, 2021, **13**, 4370-4378.

6 Z. He, L. Zhang, G. Peng, G. Wang and X. Liang, *J Anal Test.*, 2021, **5**, 350-359.

7 L. Liu, J. Du, W.-e. Liu, Y. Guo, G. Wu, W. Qi and X. Lu, *Anal. Bioanal. Chem.*, 2019, **411**, 2189-2200.

8 O. Adegoke, S. Zolotovskaya, A. Abdolvand, N. N. Daeid, *Talanta*, 2021, **224**, 121875.

9 M. H. Ibrahim, Z. Xue, H. I. Abdu, M. I. Shinger, A. M. Idris, M. M. Edris, D. Shan and X. Lu, *Nanoscale Adv.*, 2019, **1**, 1207-1214.

10 M. Wang, P. Liu, H. Zhu, B. Liu and X. Niu, *Biosensors*, 2021, **11**, 280.