Supporting Information

Morphology-Dependent Nanoplasmonic Assay: A Powerful Signaling Platform for Multiplexed Total Antioxidant Capacity Analysis

Zeinab Feyzollahi, Mahdiye Hassanpoor, Afsaneh Orouji, and Mohammad Reza Hormozi-Nezhad *

Department of Chemistry, Sharif University of Technology, Tehran, 111559516, Iran

*Corresponding authors:

- Mohammad Reza Hormozi-Nezhad, email: hormozi@sharif.edu
ORCID iD: 0000-0002-7472-1850

Table of Contents

Chemicals and Materials

Hydrogen tetrachloroaurate ($HAuCl_4.3H_2O$) (99.5% w/w), sodium borohydride ($NaBH_4$), cetyltrimethylammonium bromide (CTAB), ascorbic acid (AA), silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), ortho-phosphoric acid (85%), acetic acid (glacial) (100%), boric acid, sodium borohydride, sodium hydroxide, and N-bromosuccinimide (NBS), cysteine ($C_3H_7NO_2S$), glutathione ($C_{10}H_{17}N_3O_6S$), and uric acid ($C_5H_4N_4O_3$) were acquired from Sigma-Aldrich at analytical grade and used without further purification. Milli-Q grade water with a resistivity of 18.2 MΩ was used throughout all experimental procedures in this research.

Instrumentation

Spectrophotometric absorbance measurements were conducted using an Agilent Cary 60 spectrophotometer with 1.0 cm glass cuvettes. Images capturing color variations were taken with a Samsung A71 smartphone. Transmission electron microscopy (TEM) analysis was performed on a Zeiss EM900 microscope, operating at an acceleration voltage of 200 kV. The pH measurements and adjustments to the solutions were carried out using a Denver Instrument Model 270 pH meter equipped with a glass electrode.

Synthesis of AuNRs

AuNRs were synthesized via a seed-mediated growth method, following previously reported protocols [1]. The synthesis process involved two main steps: preparing the seed and growth solutions. For the seed solution, 0.125 mL of $HAuCl_4$ (0.01 mol L^{-1}) and 5.0 mL of CTAB (0.1 mol L^{-1}) were combined, followed by the addition of 0.3 mL of freshly prepared ice-cold $NaBH_4$ (0.01 mol L^{-1}). This resulted in a brownish-yellow solution, which was allowed to stand at room temperature for 2–5 hours. The growth solution was prepared by mixing 50.0 mL of CTAB (0.1 mol L^{-1}), 2.5 mL of $HAuCl_4$ (0.01 mol L^{-1}), and 0.3 mL of $AgNO_3$ (0.01 mol L^{-1}), followed by the addition of 0.3 mL of AA (0.1 mol L^{-1}). The color change from yellow to colorless indicated the reduction of Au^{3+} to Au^{+}. Finally, the 0.25 mL seed solution was gently added to the growth mixture, and then left overnight at room temperature. Excess CTAB was eliminated through centrifugation at 8000 rpm for 15 minutes, and the AuNRs were isolated in deionized water. The resultant AuNRs were utilized without further purification for subsequent experiments.

Statistical Analysis

Statistical analyses were performed using Origin Pro 2018 and MATLAB R2013a software. The qualitative performance of the probe was assessed using linear discriminant analysis (LDA). Partial least squares regression (PLSR) was utilized as a powerful multivariate calibration method to quantify antioxidants and total antioxidants. Principal component analysis (PCA) was applied to reduce the dimensionality of the dataset when necessary. Model accuracy was estimated using leave-one-out cross-validation and test-set validation. The Jackknifed classification matrix and canonical score plots were applied to assess the classification model outcomes. Two-dimensional (2D) confidence ellipses indicating 95% confidence limits were plotted around cluster centroids to demonstrate the statistical significance of classifications. The MVC1 toolbox in MATLAB R2019b was used for PLSR analyses, determining analytical figures of merit such as the correlation coefficient (R^2), root-mean-square error of calibration (RMSEC), root-mean-square error of cross-validation (RMSECV), root-mean-square error of prediction (RMSEP), sensitivity (SEN), analytical sensitivity (Anal. SEN), limit of detection (LOD), and limit of quantification (LOQ).

LOD and LOQ Formulas:

In this study, the LOD for each antioxidant (AA, CYS, GSH, and UA) and total antioxidant capacity (TAC) was determined using the MVC1 toolbox in MATLAB, with the results presented systematically in Table 1. The formula used to define the LOD and limit of quantification (LOQ) for multivariate calibration is provided below:

$$\mathbf{LOD = 3.3\ [SEN^{-2}\ \sigma_x^2\ (1+h_0) + h_0\ \sigma_{y,\,cal}^2\ ]^{1/2}}$$

where:

- SEN is the sensitivity,
- σ_x^2 is the instrumental signal variance,
- $\sigma_{y,cal}^2$is the variance of the analyte concentration in the calibration set,
- h_0 is the leverage of the blank sample.

The factor 3.3 corresponds to a 5% error margin for Type I and II errors. The limit of quantification (LOQ) is defined as:

$$\mathbf{LOQ = 10.0/3.3\ LOD}$$

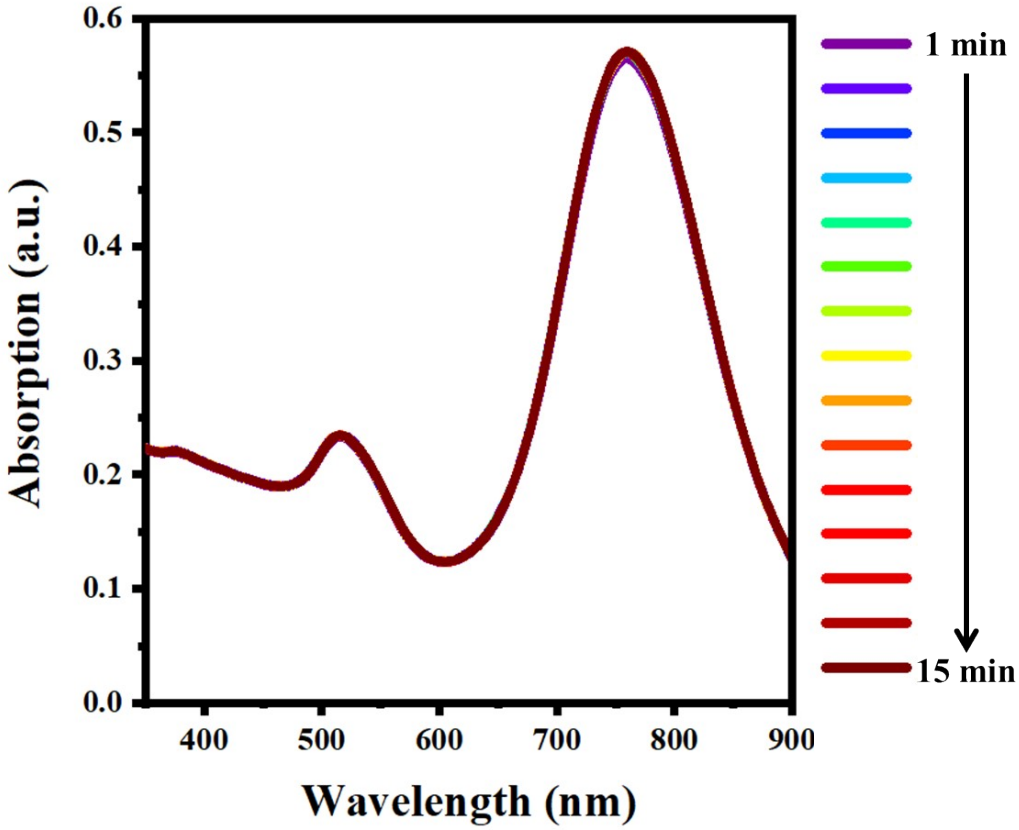

Fig. S1 Assessing the stability of AuNRs in pH 7 B.R. buffer: UV-vis spectra within 15 minutes.

Table S1. Comparative redox potentials for key antioxidants in the human body.

Redox Pair	Redox potential (volts)
Ascorbic acid oxidized/reduced	+0.08
Cystine/ Cysteine	-0.22
Glutathione oxidized/reduced	-0.24
Uric acid oxidized/reduced	0.35

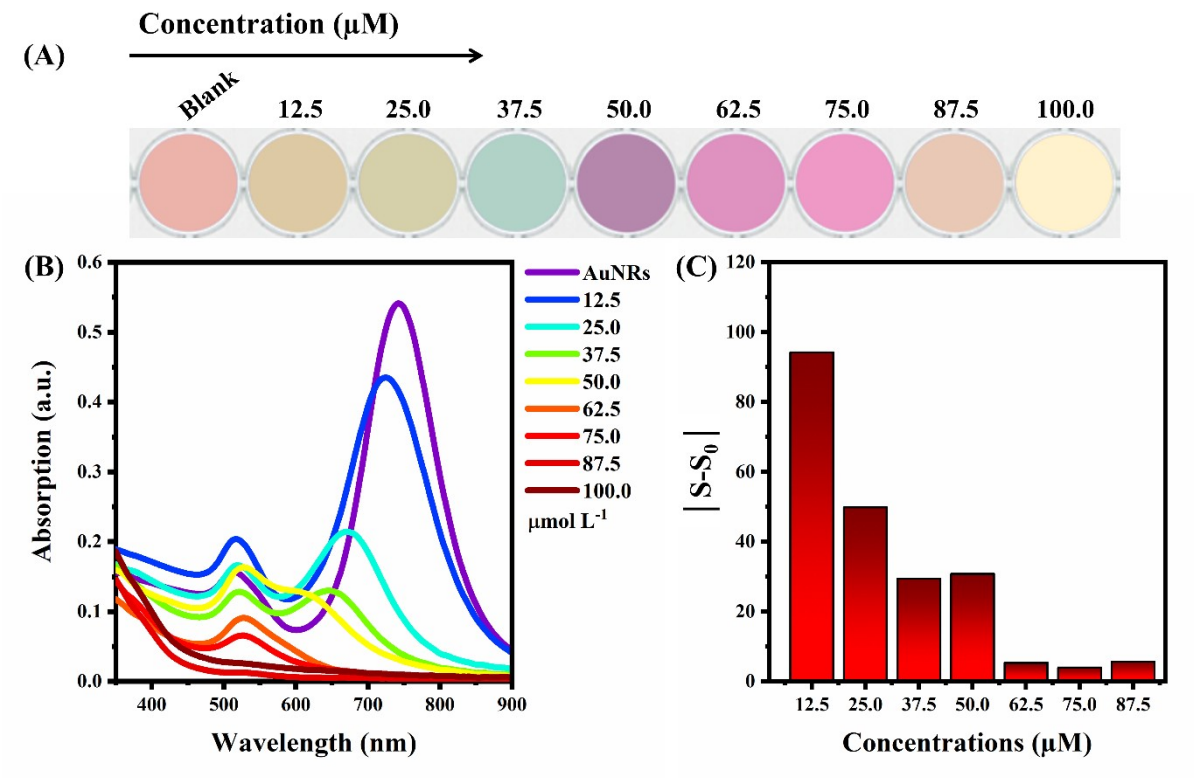

Fig. S2 Effect of NBS concentrations on the multi-colorimetric responses of AuNRs, (A) the corresponding images of the probe, (B) the absorption spectra of the proposed probe, and (C) the corresponding bar plot as a function of NBS concentration (pH 7 B.R. buffer) at 15 min in both incubation time and analysis time.

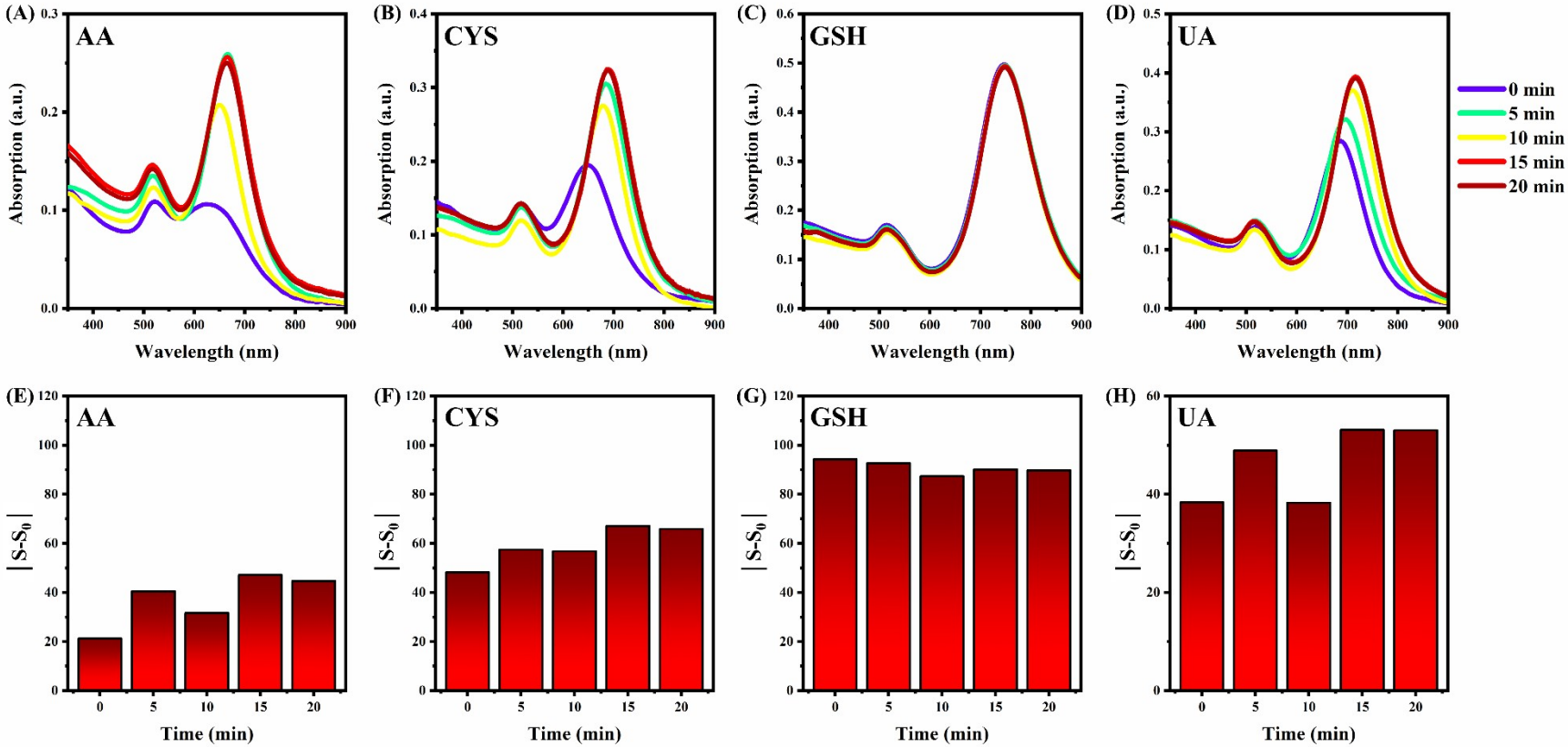

Fig. S3 Effect of incubation time on the multi-colorimetric responses of AuNRs, spectral variations of 10 µM of each antioxidant, pH 7 B.R buffer, and analysis time 15 min (A) AA, (B) CYS, (C) GSH, and (D) UA, (E-H) bar plots representing variations of AuNRs spectra as a function of incubation time in the presence of AA, CYS, GSH, and UA respectively.

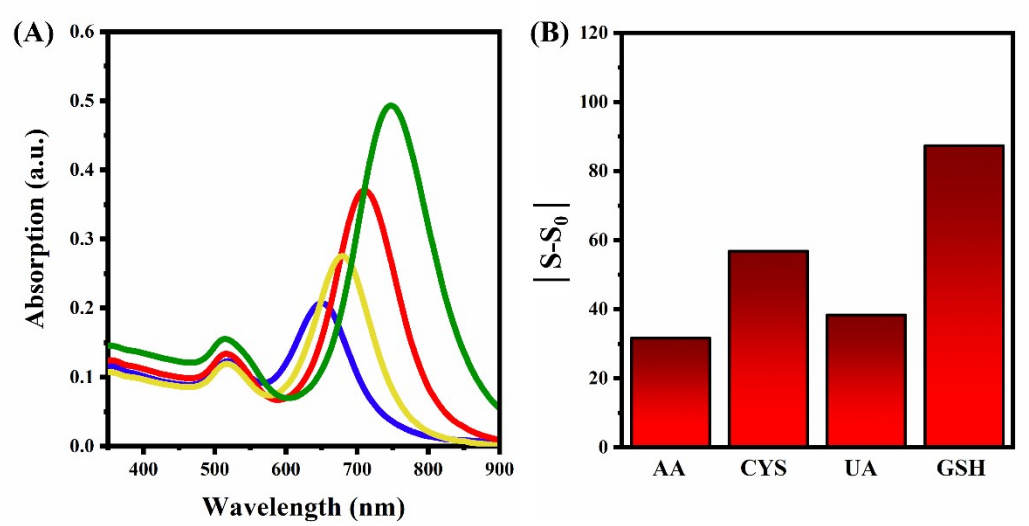

Fig. S4 UV-vis absorption profile of the proposed probe after 10-minute incubation with 75 μM NBS and 10 μM of individual antioxidants in pH 7 B.R. buffer.

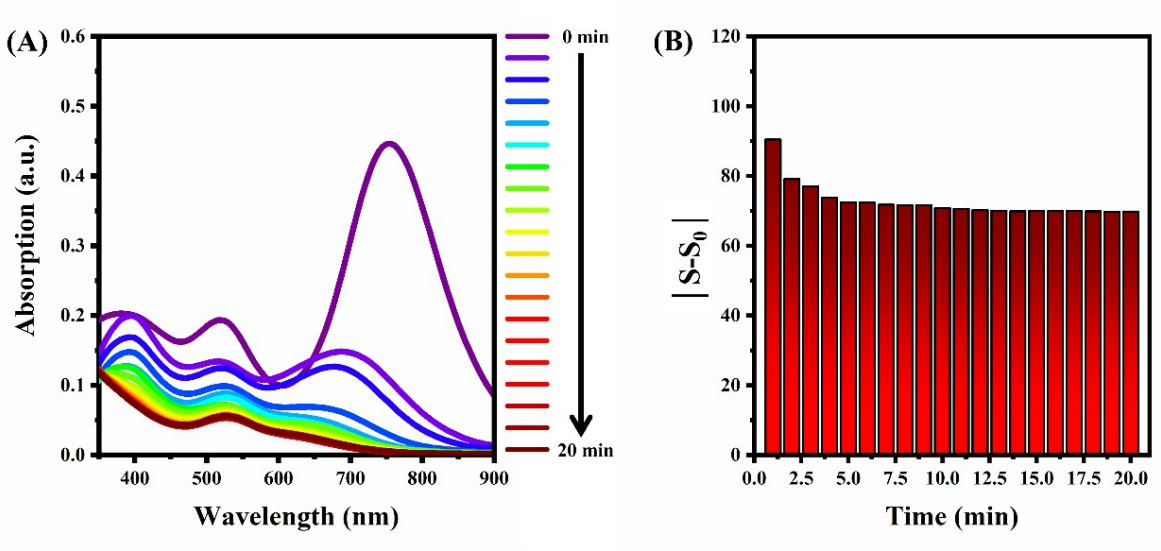

Fig. S5 Effect of time-course variation in absorption spectra of the AuNRs, (A) spectral variations, (B) bar plot representing variations of AuNRs spectra as a function of time-course variation in the presence of 75 μM NBS. The incubation time was 10 min.

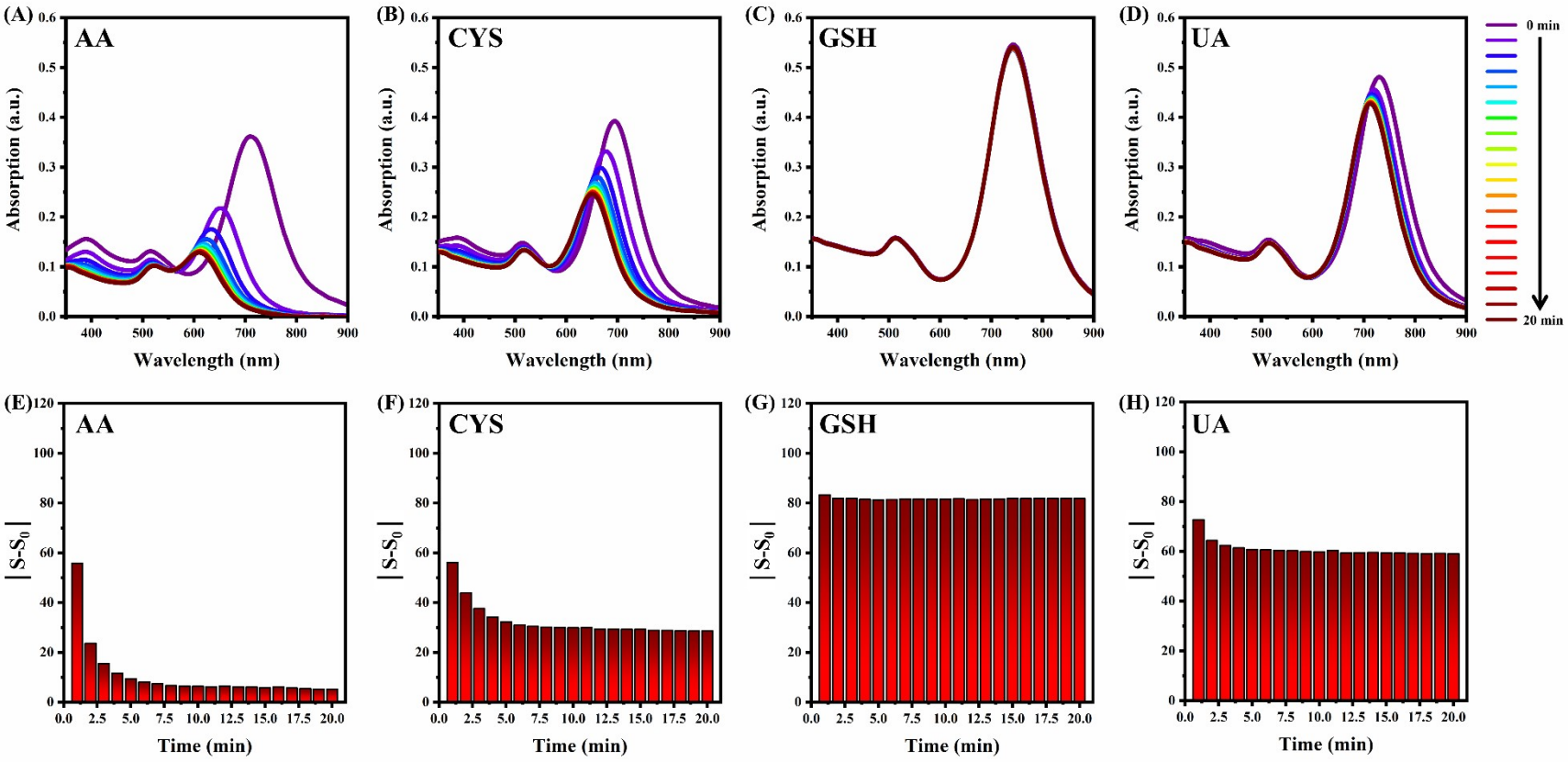

Fig. S6 Effect of time-course variation in absorption spectra of the AuNRs, spectral variations of (A) ASA, (B) CYS, (C) GSH, and (D) UA, (E-H) bar plots representing variations of AuNRs spectra as a function of time-course variation in the presence of AA, CYS, GSH, and UA, respectively. The concentration of NBS and each antioxidant was 75 μmol L^{-1} and 10 μmol L^{-1}, respectively. The incubation time was 10 minutes in pH 7 B.R. buffer.

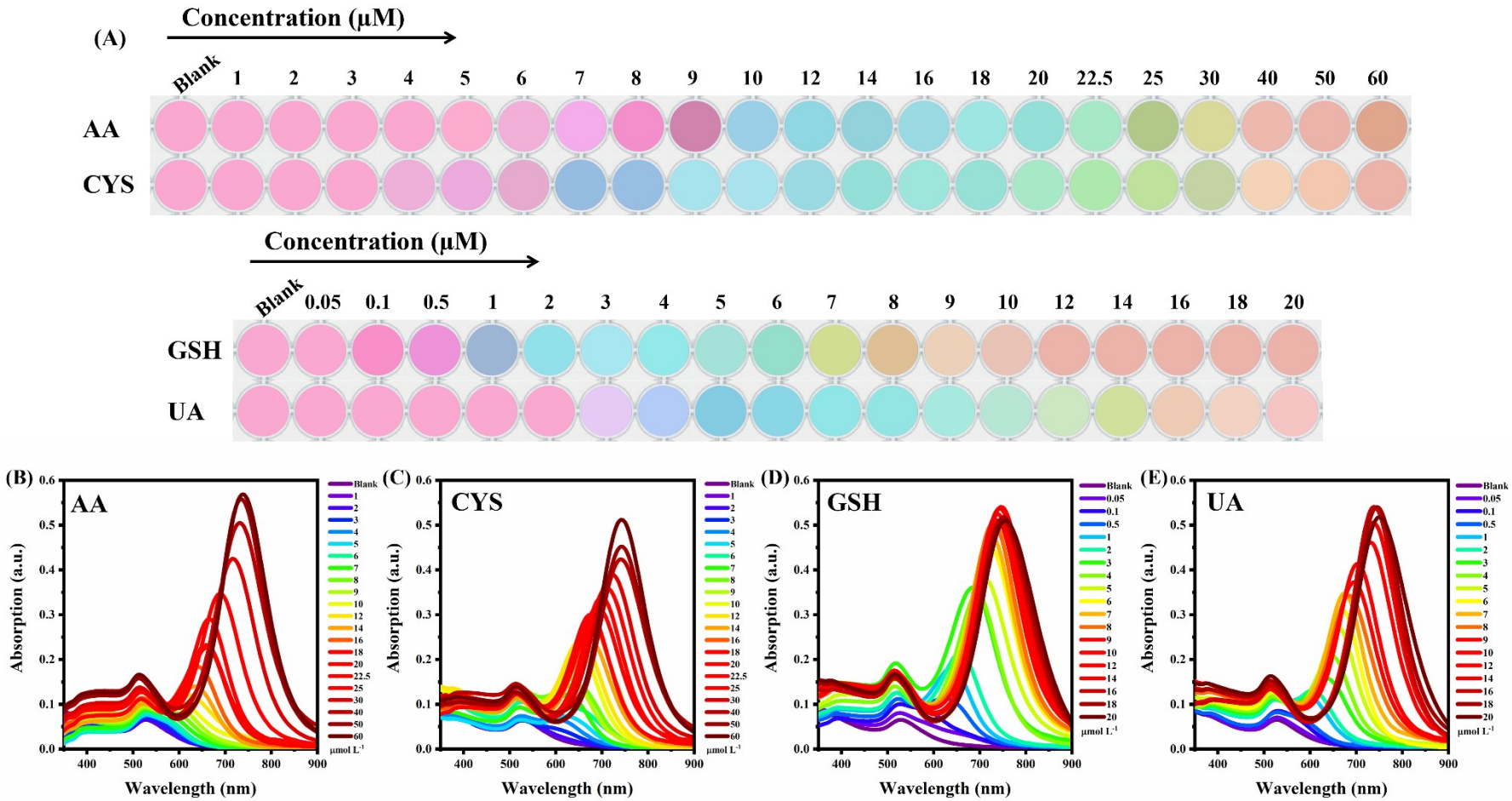

Fig. S7 (A) Color variation images and variation responses of the probe to different concentrations of (B) AA, (C) CYS, (D) GSH, and (E) UA.

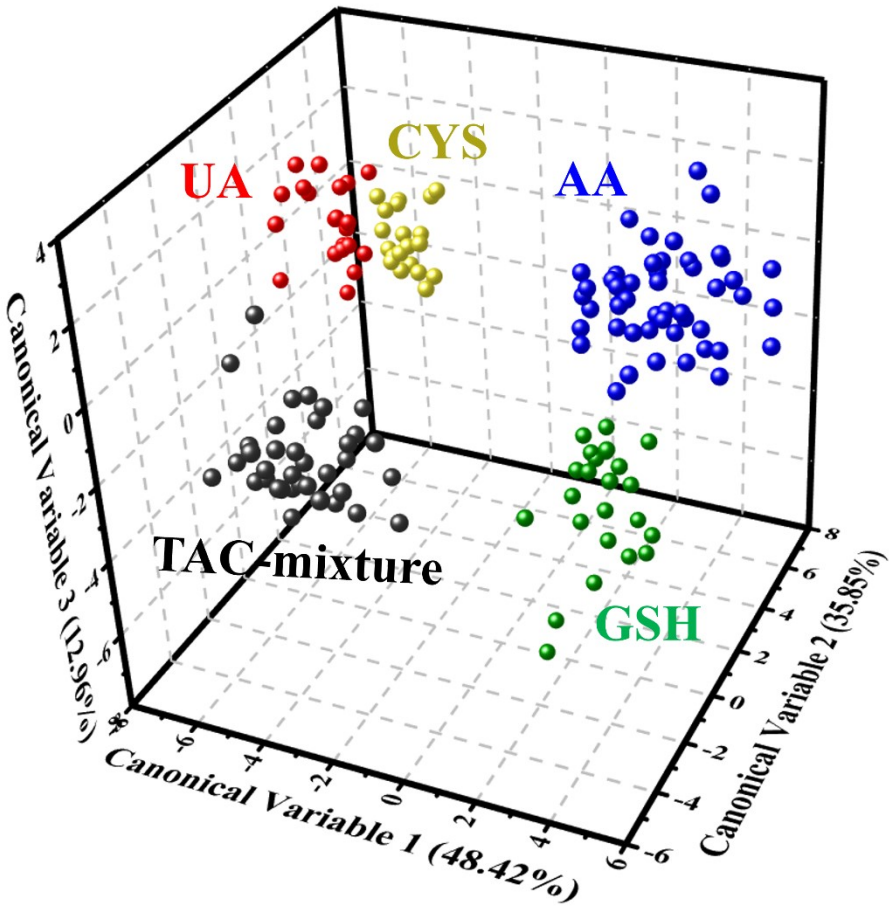

Fig. S8 3D LDA score plots for the discrimination of four classes of antioxidants (i.e., AA, CYS, GSH, and UA) and total antioxidants as a TAC-mixture.

Table S2. Jackknifed classification matrix for the discrimination of single-component samples (AA and CYS 1-60 μmol L^{-1}; UA and GSH 0.05-20 μmol L^{-1}) and TAC-mixture as a multicomponent (0.05-14 μmol L^{-1}) in their entire concentration range.

Nominal class	Predicted class: AA	CYS	GSH	UA	TAC-mixture	Total	Sensitivity (%)	Specificity (%)	Precision (%)
AA	51	0	0	0	0	51	100.00	100.00	100.00
	100.00%	0.00%	0.00%	0.00%	0.00%	100.00%			
CYS	0	21	0	0	0	21	100.00	100.00	100.00
	0.00%	100.00%	0.00%	0.00%	0.00%	100.00%			
GSH	0	0	21	0	0	21	100.00	100.00	100.00
	0.00%	0.00%	100.00%	0.00%	0.00%	100.00%			
UA	0	0	0	24	0	24	100.00	100.00	100.00
	0.00%	0.00%	0.00%	100.00%	0.00%	100.00%			
TAC-mixture	0	0	0	0	36	36	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	100.00%	100.00%			
Total	51	21	21	24	36	1153	100.00	100.00	100.00
	33.33%	13.73%	13.73%	15.69%	23.53%	100.00%			

Table S3. Jackknifed classification matrix for the discrimination of AA in the entire concentration range.

Nominal class \ Predicted class	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22.5	25	30	40	50	60	Total	Sensitivity (%)	Specificity (%)	Precision(%)
1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
4	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
5	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
6	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
8	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
9	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
10	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
12	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
14	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
16	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
18	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
22.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	4.76%			
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	4.76%			
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	4.76%			
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	4.76%			
60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	4.76%			
Total	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	63	100.00	100.00	100.00
	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	4.76%	100.00%			

Table S4. Jackknifed classification matrix for the discrimination of CYS in the entire concentration range.

Table S5. Jackknifed classification matrix for the discrimination of GSH in the entire concentration range.

Nominal class	Predicted class: 0.05	0.1	0.5	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	Total	Sensitivity (%)	Specificity (%)	Precision(%)
0.05	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
0.1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
0.5	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
3	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
4	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
5	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
6	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
7	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
8	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
9	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
10	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
12	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	7.41%			
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	7.41%			
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	7.41%			
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	7.41%			
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	7.41%			
Total	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	54	100.00	100.00	100.00
	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	7.41%	100.00%			

Table S6. Jackknifed classification matrix for the discrimination of UA in the entire concentration range.

Table S7. Jackknifed classification matrix for the discrimination of TAC-mixture in the entire concentration range.

Nominal class	Predicted class: 0.05	0.1	0.5	1	2	3	4	5	6	7	8	9	10	12	14	Total	Sensitivity (%)	Specificity (%)	Precision(%)
0.05	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
0.1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
0.5	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
3	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
4	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
5	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
6	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
7	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
8	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	6.67%			
9	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	6.67%			
10	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	6.67%			
12	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	6.67%			
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	100.00	100.00	100.00
	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	6.67%			
Total	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	45	100.00	100.00	100.00
	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	6.67%	100.00%			

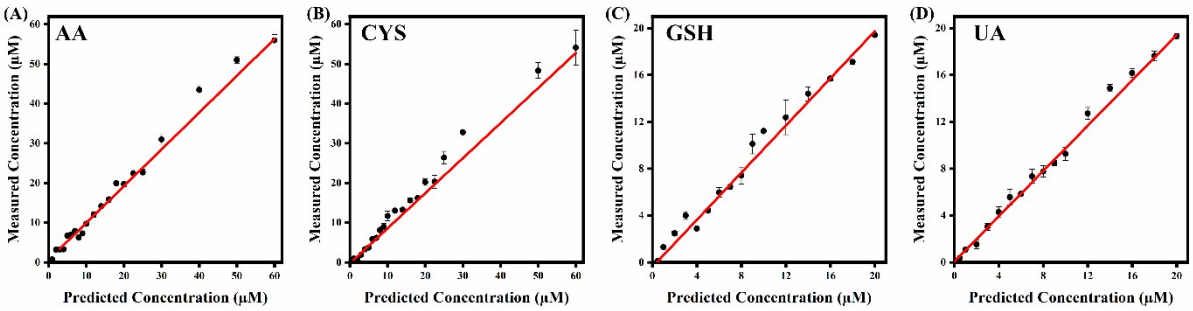

Fig. S9 Predicted vs measured concentration plots with PLSR for (A) AA, (B) CYS, (C) GSH, (D) and UA.

Table S8. Comparison of the proposed method with other reported methods for detection of antioxidants.

Analytes	Materials	Detection method	Linear range (μmol L^{-1})	LOD (μmol L^{-1})	References
AA CYS GSH	Iron-doped carbon nitride	Smartphone-based colorimetric	50-1000 5-22.5 5-30	30 4 8	[1]
AA CYS GSH	Ag-MOF	Visual-based	3-40	0.048 0.050 0.088	[3]
TAC	SMOF nanozyme	Colorimetric	50−700	33.4	[4]
AA CYS GSH	Mn-MOF peroxidase-like nanozymes	Temperature-resilient	3.0-25 3.0–33 3.0-35	0.040 0.047 0.067	[5]
TAC	Au-doped g-C3N4 nanosheet	Colorimetric	Not reported	1.0	[6]
AA	Nitrogen-Doped Carbon Nanoflowers	Colorimetric	1.0−20.0	0.94	[7]
GSH CYS GA CA	Au_2Pt nanozymes	Colorimetric sensor array	4–20 0-16 1-20 2-12	0.124 0.1163 0.2570 0.1885	[8]
AA CYS GSH UA TAC	Anti-etching of AuNRs	Colorimetric	3.1-60.0 2.6-60.0 1.2-20.0 0.8-14.0 0.7-14.0	1.1 0.9 0.4 0.3 0.2	This Work

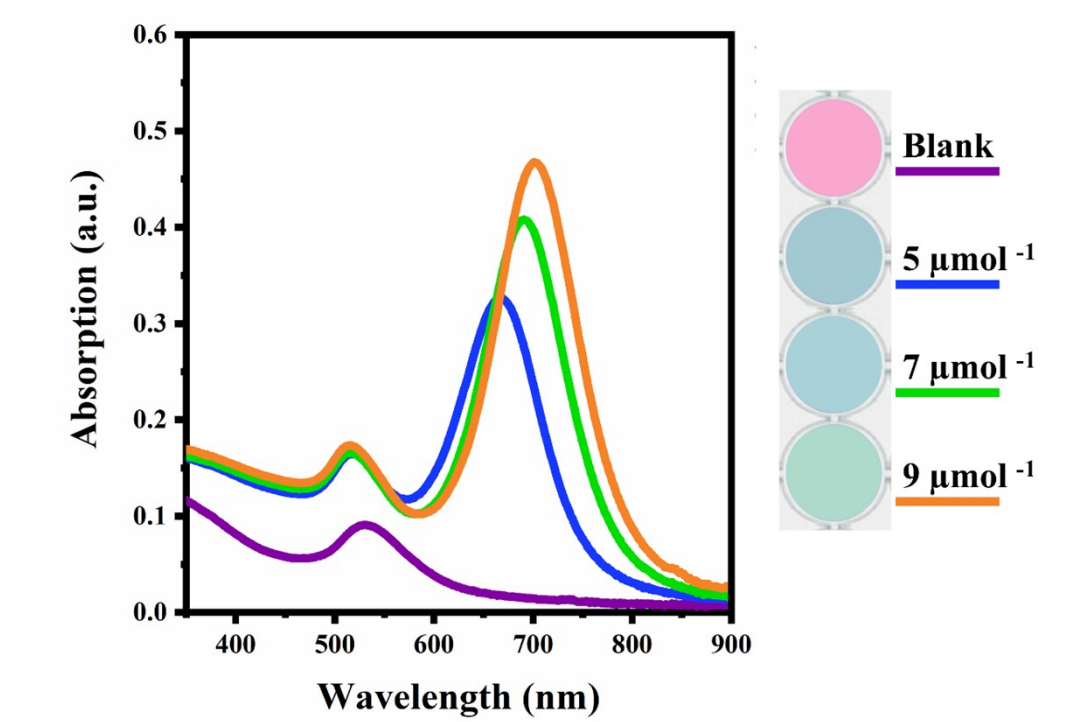

Fig. S10 UV-vis spectra and corresponding color photographs of three different TAC unknown in total concentration of 5, 7, and 9 μmol L^{-1} in human saliva sample.

Table S9. LDA posterior probability outcomes for identifying TAC unknown samples in human saliva. All 9 samples were given as a test set to the pre-trained LDA model of AA, CYS, GSH, UA, and TAC-mixture.

Alleged	***Post probabilities***					***Allocated***
	AA	***CYS***	***GSH***	***UA***	***TAC-mixture***	
TAC-mixture	5.44E-14	3.24E-18	4.75E-20	9.29E-18	1	TAC-mixture
TAC-mixture	1.83E-12	3.84E-9	9.72E-15	5.54E-7	1	TAC-mixture
TAC-mixture	2.90E-13	4.43E-10	1.33E-15	1.53E-8	1	TAC-mixture
TAC-mixture	2.50E-13	4.99E-27	2.36E-16	7.11E-28	1	TAC-mixture
TAC-mixture	2.92E-13	2.63E-38	8.17E-19	1.89E-42	1	TAC-mixture
TAC-mixture	3.36E-13	2.52E-23	9.71E-16	1.72E-23	1	TAC-mixture
TAC-mixture	1.82E-7	2.85E-4	0.04335	1.78E-4	0.95619	TAC-mixture
TAC-mixture	7.97E-21	1.34E-27	3.85E-15	2.03E-32	1	TAC-mixture
TAC-mixture	1.25E-10	1.23E-8	2.60E-6	1.64E-10	1	TAC-mixture

Table S10. LDA posterior probability outcomes for identifying TAC unknown samples in human saliva. All 9 samples were given as a test set to the pre-trained LDA model of TAC-mixture within the total concentration range of 0.05–14 µmol L^{-1}.

Alleged	***Post probabilities***															***Allocated***
	0.05	***0.1***	***0.5***	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***12***	***14***	
5	0	0	0	0	0	0	0	1	3.39E-92	1.65E-58	0	0	0	0	0	5
5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	5
5	0	0	0	0	0	0	0	1	0	1.82E-75	0	0	0	0	0	5
7	0	0	0	0	0	0	0	1.02E-179	3.76E-73	1	6.43E-127	4.65E-82	0	0	0	7
7	0	0	0	0	0	0	0	1.13E-153	8.36E-64	1	1.44E-145	1.20E-111	0	0	0	7
7	0	0	0	0	0	0	0	1.42E-136	2.18E-53	1	7.02E-139	9.23E-117	0	0	0	7
9	0	0	0	0	0	0	0	0	3.58E-265	1.18E-195	1.80E-80	1	0	0	0	9
9	0	0	0	0	0	0	0	0	9.28E-234	7.21E-165	3.29E-77	1	0	0	0	9
9	0	0	0	0	0	0	0	0	3.96E-246	2.83E-178	2.72E-77	1	0	0	0	9

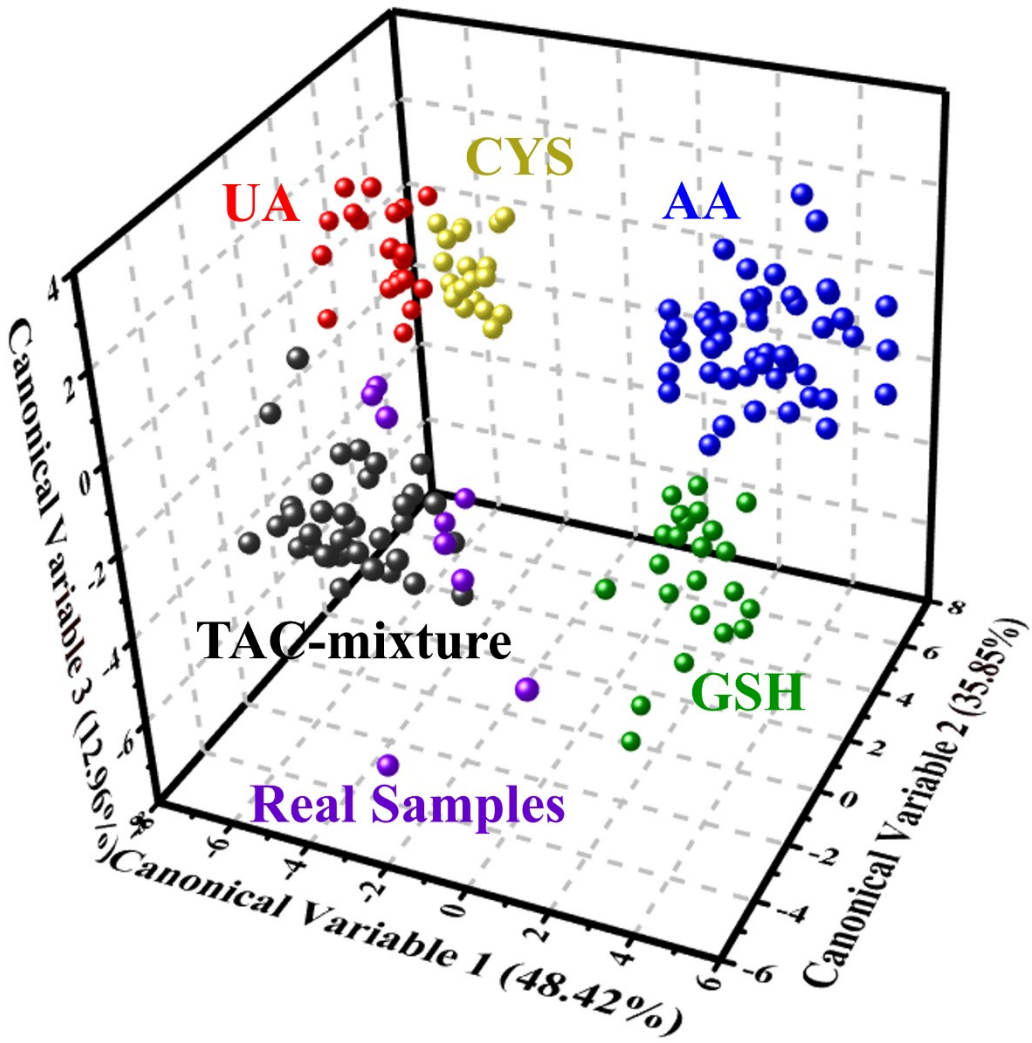

Fig. S11 3D LDA score plots for the discrimination of TAC unknown of real human saliva samples after combining the test set (real samples) with the pre-trained LDA model of AA, CYS, GSH, UA, and TAC-mixture. The saliva samples were spiked with three concentrations of total antioxidant.

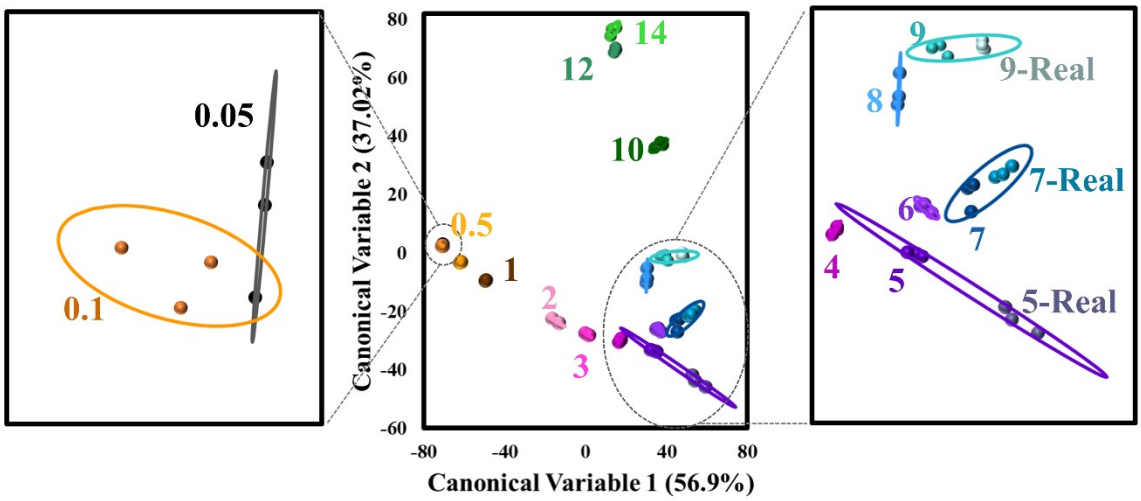

Fig. S12 2D LDA score plots for the discrimination of TAC unknown of real human saliva samples after combining the test set (real samples) with the pre-trained LDA model of TAC-mixture within the total concentration range of 0.05–14 μmol L^{-1}. The saliva samples were spiked with three concentrations of total antioxidants.

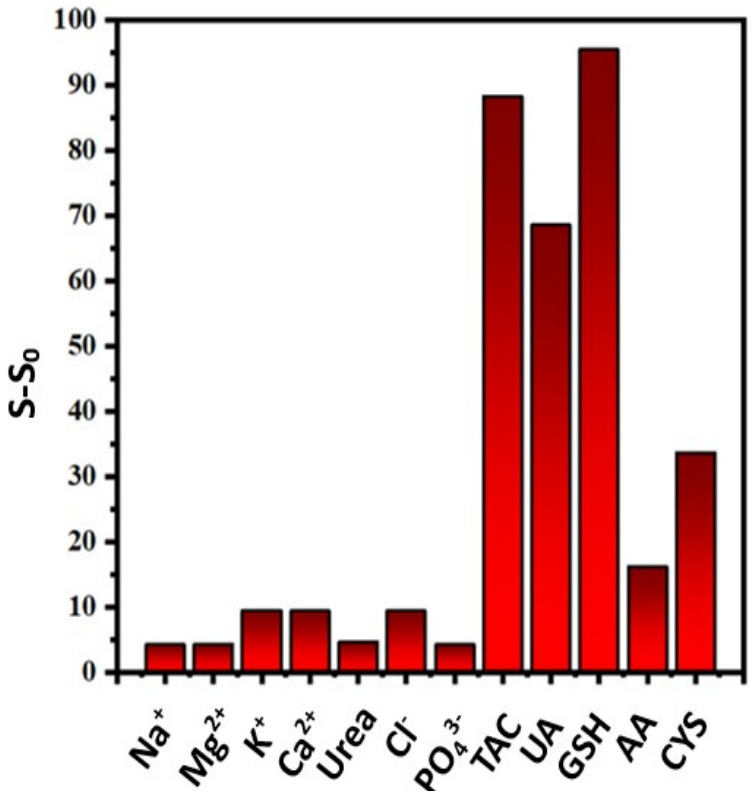

Fig. S13 Interference effect on the responses of the proposed probe ([AA] = [CYS] = [GSH] = [UA] = [TAC] = 10 μM, [Urea] = 475 μM, ([Na^+] = 1000 μM, [Ca^{2+}] = [K^+] = 500 μM, [Mg^{2+}] = 650 μM) ([Cl^-] = 650μM, [PO_4^{3-}] = 375 μM).

References

1. M. R. Hormozi-Nezhad, H. Robatjazi and M. Jalali-Heravi, *Anal. Chim. Acta*, 2013, **779**, 14-21.
2. Y. Miao, M. Xia, C. Tao, J. Zhang, P. Ni, Y. Jiang and Y. Lu, *Talanta*, 2024, **267**, 125141.
3. S. S. Mohammed Ameen and K. M. Omer, *ACS Appl. Nano Mater.* 2024, **7**, 20793-20803.
4. H. Wu, J. Chen, P. Lin, Y. Su, H. Li, W. Xiao and J. Peng, *ACS Appl. Mater. Interfaces.* 2024, **16**, 39857-39866.
5. S. S. M. Ameen and K. M. Omer, *Food Chem*. 2024, **448**, 139170.
6. X. Li, W. Fan, H. Tang, D. Li, Y. Xiao, B. Yang, Y. Zhao and P. Wu, *Food Chem.* 2024, **439**, 138158.
7. Y.-W. Mao, J.-Q. Li, R. Zhang, A.-J. Wang and J.-J. Feng, *ACS Appl. Nano Mater.* 2023, **6**, 2805-2812.
8. F. Wu, H. Wang, J. Lv, X. Shi, L. Wu and X. Niu, *Biosens. Bioelectron.* 2023, **236**, 115417.