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**Research Report**

**The Team**

Registration Number: Chem-040

Name of team member: YU Xinjie

School: Hwa Chong Institution

City, Country: Singapore, Singapore

Name of supervising teacher: CHEN Zhengbo

Position: Associate Professor

School/Institution: Capital Normal University

City, Country: Beijing, China

**Title of Research Report**

Colorimetric discrimination of multiple antioxidants based on three chloroauric acid/core-shell Au@Ag nanocube sensors

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# Colorimetric discrimination of multiple antioxidants based on three chloroauric acid/core-shell Au@Ag nanocube sensors

## YU Xinjie

## Abstract

Antioxidant species are crucial to normal biological functions, yet inappropriate doses of certain species have deleterious effects on human health. In this study, a colorimetric sensor array for the discrimination of multiple antioxidants has been developed. In order to extract data-rich colorimetric responses from the sensor array, Au@Ag nanocubes (NCs) with a core-shell structure as colorimetric probes were rationally prepared, and three different concentrations of chloroaurate acid (HAuCl$_4$) were employed as sensing elements. Interestingly, Au$^{3+}$ ions can be reduced to different valence states (i.e., Au(0) and Au(I)) by different antioxidants, thus effectively inhibited the oxidation etching process of Au@Ag NCs by Au(III) ions to varying extents, generating simultaneous colorimetric signal output to enable identification of the six antioxidants at 10 nM via linear discriminant analysis (LDA). The discrimination ability of the sensor array was further evaluated in antioxidant mixtures from binary to component mixture. Remarkably, identification of these six antioxidants spiked in urine was also realized with 100% of accuracy.

**Keywords**：Au@Ag nanocubes, sensor array, silver etching, antioxidant, linear discriminant analysis

**Acknowledgement**

I would like to express my gratitude to my mentor, Dr. Chen Zhengbo for his guidance and support, without which this research project would not be possible.

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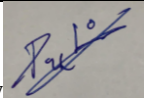
*(Signatures of full team below)*

Name of team member: Yu Xinjie

Name of supervising teacher: Chen Zhengbo

Noted and endorsed by

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# 1. Introduction

In normal cellular metabolism, reactive oxidative species (ROS) are often produced. However, excess ROS lead to oxidative stress in biological systems. Under high oxidative stress, biomolecules vital to cellular processes undergo structural or functional changes, ultimately resulting in tissue damage[1]. Consumption of antioxidants has been shown to help restore the biochemical balance between ROS and cellular antioxidative activity. Specific antioxidants, in due amounts, are able to prevent complex diseases including diabetes, stroke, Parkinson's disease, cardiovascular disorders, inflammatory diseases, and cancer[2]. For instance, dopamine (DA) has been shown to resist shock. Citric acid (CA) can reduce the risk of cardiovascular arteriosclerosis by regulating blood viscosity. Inappropriate use of other antioxidants, however, is demonstrated to cause cancer and even life loss[3,4]. Hence, it is desirable to develop a reliable and versatile method for determination and discrimination of antioxidants.

While there has not been a standardised method to determine antioxidant capacity, current *in-vitro* methods can be classified into two broad categories consisting of single electron transfer (ET)-based assays and hydrogen atom transfer (HAT)-based assays[5]. Common ET-based assays include the Folin-

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Ciocalteu reagent[6,7], Ferric reducing-antioxidant power[8,9] (FRAP), Cupric ion reducing antioxidant capacity [10] (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl [11] (DPPH), and ABTS/trolox equivalent antioxidant capacity [12] (TEAC). Common HAT-based assays include oxygen radical absorbance capacity [13][14][15][16] (ORAC) and total radical trapping antioxidant parameter [17] (TRAP). All of these methods require large reagent and sample volumes, and thus require cumbersome, labour intensive

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sample preparation and handling. It should be emphasized that these methods are difficult to achieve high-throughput screening of multiple antioxidants.

Colorimetric assays provide new opportunities for distinguishing and determining antioxidant species due to its simplicity and practicality. Such methods do not require sophisticated equipment and produce results observable by the naked eye. Various colorimetric methods based on etching and subsequent aggregation of nanoparticles (NPs) have emerged. However, NP-based methods often suffer from poor specificity due to the similar oxidation susceptibility among antioxidants[18]. An improved assay would retain the ease of operation and also exhibit high degree of specificity. The array sensing technique proves such improvement possible. Rather than the conventional "lock-key" interaction between sensor-target couples [19], drawing inspiration from the mechanisms of mammalian olfactory/gustatory responses [20], sensor arrays generate multiple responses based on the cross-responsiveness of a series of receptors to a particular target.

In this study, we report a novel colorimetric sensor array for discrimination of six types of antioxidants (i.e., citric acid (CA), tartaric acid (TA), ascorbic acid (AA), tea polyphenol (TP), tannic acid (TA), and gallic acid (GA)). In this design, three concentrations of $HAuCl_4$ were employed as sensor receptors, and core-shell Au@Ag nanocubes (NCs) served as colorimetric probes. As we know, Ag shell is etched by $Au^{3+}$ and induces a colour change. However, the presence of various antioxidants inhibits the oxidation activity of $Au^{3+}$ to different extents. Consequently, the Au@Ag NCs are etched to diverse degrees upon the introduction of different antioxidants due to the reaction between antioxidant and $Au^{3+}$ and exhibit differential colorimetric responses. The response pattern as a fingerprint is unique to each analyte and can be identified by linear discriminant analysis (LDA).

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# 2. Experiment Section

## 2.1 Chemicals

HAuCl₄, AgNO₃, NaBH₄, ascorbic acid (AA), cetyltrimethylammonium bromide (CTAB), and cetyltrimethylammonium chloride (CTAC) were obtained from Sigma-Aldrich. Citric acid (CA), tartaric acid (TA), ascorbic acid (AA), tea polyphenol (TP), tannic acid (TA), and gallic acid (GA) were purchased from Aladdin (Shanghai, China). Ultrapure water (18.2 MΩ/cm) was acquired from a Mili-Q ultrapure system. All the reagents were analytical grade.

## 2.2 Apparatus

Absorption values were recorded on SpectraMax$^R$M2e Multi-Mode Microplate Reader (Molecular Devices, California, USA). Transmission electron microscopy (TEM) images were performed on a JEM-2100 microscope at an acceleration voltage of 200 kV (JEOL, Japan). The absorption of the 96-well plate at 425 nm was collected by a SpectraMax M2e microplate reader (Molecular Devices, U.S.A.).

## 2.3 Preparation of Au@Ag NCs

The preparation process of Au@Ag NCs can be found in previous literature[21]. The synthesis is divided into three steps: the first step is to synthesize Au seeds, the second step is to synthesize CTAC-capped Au seeds, and the third step is to synthesize Au@Ag NCs. The whole preparation process is as follows: We first mixed 5 mL of 0.25 mM HAuCl4 and 5 mL of 100 mM CTAB. 0.6 mL of 10 mM ice cooled NaBH4 was then added into the solution. The yellow solution turned colourless upon addition. An eventual light red colouration was obtained after 3 h, indicating the formation of 3 nm Au seeds.

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The complete dissolution of NaBH4 was ensured by keeping the 3 nm Au seed solution at room temperature for 3 h. Subsequently, 4.5 mL of 100 mM AA was added to 12 mL of the mixture composed of 6 mL of 0.5 mM HAuCl4 and 6 mL of 0.2 M CTAC, followed by the addition of 0.3 mL of 3 nm Au seeds. A colour change from colourless to claret was observed. After resting 1 h, the suspension of Au nanocrystals was centrifuged at 15000 rpm for 30 min. Then the Au nanocrystals were washed thoroughly with water and dispersed in deionized water. Next, 0.5 mL of the previous CTAC-Au seeds and 4.5 mL of 20 mM CTAC were mixed. The mixture was heated at 60 °C for 20 min under magnetic stirring. 2 mL of 2 mM AgNO3 and a mixture of 1 mL of 50 mM AA and 1 mL of 40 mM CTAC were simultaneously added using a syringe pump. The colour changed from red to brown. Stirring was continued at 60 °C for 4 h and the resulting mixture was cooled in an ice-bath. Finally, the product was separated by centrifugation at 15000 rpm for 15 min. The resulting Au@Ag NCs were washed with deionized water three times and stored at 4 °C before use.

## 2.4 Discrimination procedure of different antioxidants

Firstly, 100 μL of three concentrations (40, 60, and 80 μM) of $HAuCl_4$ and 370 μL of Au@Ag NC solution were well mixed in phosphate buffered saline (PBS) (pH=7.0) to form three $HAuCl_4$/Au@Ag NCs sensors (40 μM $HAuCl_4$/Au@Ag NCs, 60 μM $HAuCl_4$/Au@Ag NCs, and 80 μM $HAuCl_4$/Au@Ag NCs). Then, 100 μL of target antioxidants (CA, TA, AA, TP, TA, and GA) were individually injected to the above sensor solutions for 30 min of incubation. Next, 100 μL of the above solutions were transferred into 96-well plates, and then the absorption of each well at 425 nm was measured by a microplate reader. Therefore, the six antioxidants were measured against the three $HAuCl_4$/Au@Ag NCs sensors 5 times to give a training data matrix of 6 antioxidants × 3 sensors × 5 replicates.

# 3. Results and discussion

## 3.1 Discrimination principle

The sensing strategy of the method is presented in Scheme 1, three sensing elements (40, 60, and 80 µM HAuCl$_4$) are first formed to fabricate the sensor array. In the absence of target antioxidants, galvanic exchange occurs between Au@Ag NCs and Au(III), Ag of the Au@Ag NCs is etched, meanwhile, Au(III) ions are reduced to Au(0) and deposited onto the surface of Au@Ag NCs, generating changes of colour and absorbance intensity. Whereas in the presence of different antioxidants, Au(III) can be reduced to other valence states, such as its zero and mono valence state, which effectively inhibits the oxidation etching process of Au@Ag NCs to varying degrees. Consequently, diverse colour and absorbance changes as "fingerprint" can be observed after the addition of antioxidants, and these gathered colorimetric signals from the sensor array can offer a map of a discriminative fingerprint by linear discriminant analysis (LDA)[22]. Therefore, an effective discrimination of the six antioxidants (i.e., CA, TA, AA, TP, TCA, and GA) can be achieved.

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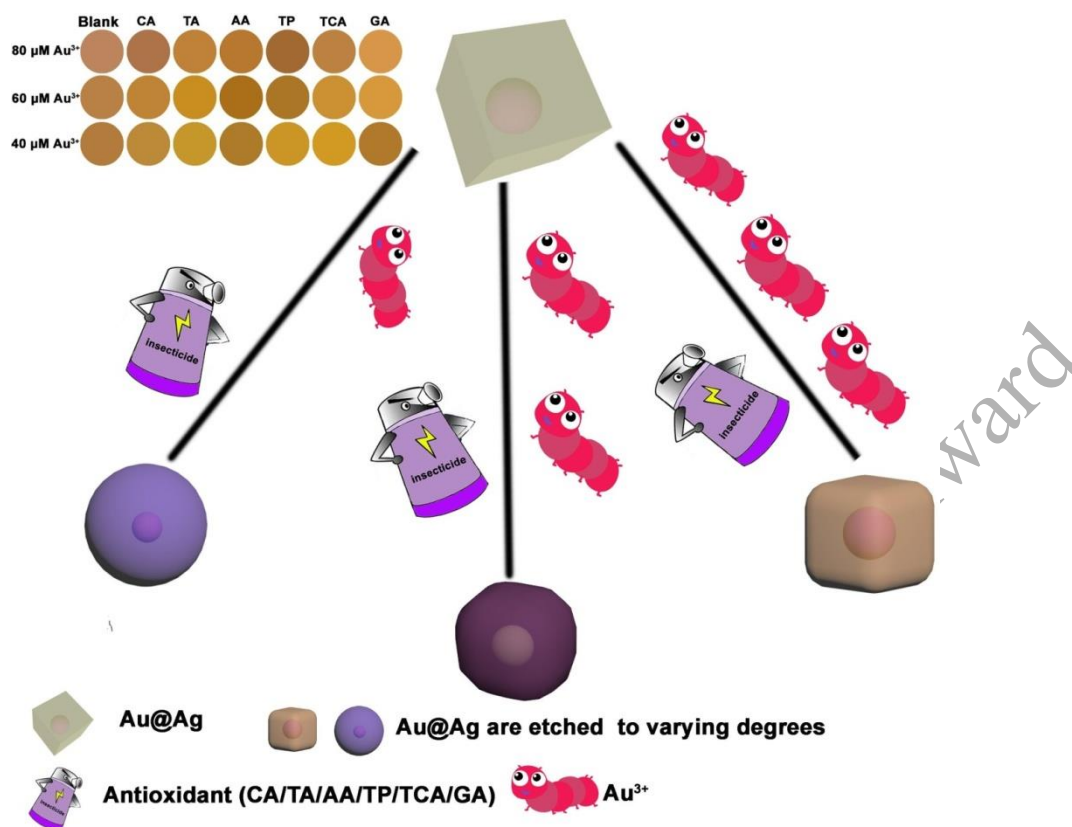
Scheme 1 Schematic illustration of the colorimetric discrimination of multiple antioxidants based on the etching of the Au@Ag NCs

## 3.2 Characterization of the Au@Ag NCs

The prepared Au@Ag NCs in the experiments were characterized by transmission electron microscopy (TEM) images. As shown in Fig. 1A, From the figure, we can clearly see that the nanoparticles have an obvious core-shell structure. HRTEM results (inset of Fig. 1A) verifies that the outer shell of the nanoparticle is Ag. The resulting elemental map ((inset of Fig. 1A) of the nanoparticles reveal that Au element was concentrated at the centre of the structure as the "core" and Ag element is distributed in the periphery of the structure as the "shell".  In addition, in the presence of antioxidants, as shown in Fig. 1(B,C), Au@Ag NCs were etched by $Au^{3+}$ to different degrees, which is attributed to the inhibitory effect of different antioxidants on Ag etching.
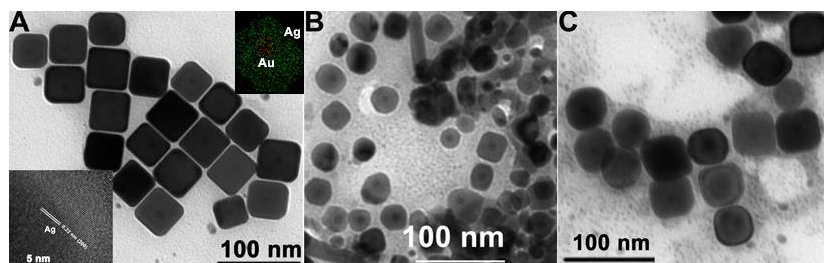
Figure 1 TEM image of Au@Ag NCs. Inset: HRTEM and element mapping. (B,C) TEM images of Au@Ag NCs after etching in the presence of different antioxidants.

## 3.2 Optimization of sensing conditions

To improve the effectiveness of the sensor array, several key parameters including pH and $Au^{3+}$ concentration were optimised. The effect of solution pH on the absorbance was first investigated. As shown in Fig.S1, as the pH value varies in the range between 6.0 and 8, the absorption peak at 425 nm decreases. When pH=7.2, the absorbance at 425 nm decreases dramatically with the appearance of the peak at 450 nm. In consideration of the inhibitory effect of antioxidant (here AA) on Au@Ag NC etching, we chose 7.0 as the optimum pH. $HAuCl_4$ concentration, as array's sensing element, has a great influence on the performance of the sensor array. We investigated the effect of 8 $HAuCl_4$ concentrations (10, 20, 40, 60, 80, 100, 150, and 200 μM) on the absorbance of the solution. As depicted in Fig. S2, about 40, 60, and 80 μM $HAuCl_4$,, in which the absorption peaks at three different wavelengths appear, corresponding to different colours. Because the sensor array must consider differences in the recognition ability of sensing elements, we chose 40, 60, and 80 μM $HAuCl_4$ as the optimal concentrations of $HAuCl_4$.

## 3.3 Array's discrimination performance

To test the discrimination ability of the sensor array, sensing behaviours of the sensor array to six antioxidants were conducted. In order to better illustrate the colorimetric responses of the sensor array, absorbance change ($\Delta A= A_{i,425\ nm}-A_{0,\ 425\ nm}$) of each antioxidant was recorded, where $A_i$ and $A_0$ represented the absorbance values of the Au@Ag NCs at 425 nm in the presence and absence of the targets, respectively. Each test was repeated five times. As shown in Fig.2, the colour map, acting as "fingerprints", clearly shows diverse changes between 6 antioxidants at the same concentration (10 nM), revealing the feasibility for antioxidant discrimination by the sensor array. To further investigate the colorimetric responses of the sensor array, linear discriminant analysis (LDA) was performed on the training matrices. The matrices were transformed into canonical factors and visualised in a three-dimensional (3D) diagram. As depicted in Fig. 3($A_1$-$F_1$), the six antioxidants at each concentration (200, 150, 100, 50 20, and10 nM) were located in six separate positions without any overlap. The discrete distribution of the six groups in the 3D LDA plot demonstrates that these six antioxidants could be well distinguished. In order to reveal the interaction pattern of each sensing elements and the target antioxidants, a "radar" pattern for the six antioxidants with the same concentration by the sensor array were formed as shown in Fig. 3($A_2$-$F_2$), it clearly shows that a discernible discrepancy exists between the interaction patterns of each sensing elements and each antioxidants with the same concentration, like the colour map, further confirming the feasibility for antioxidant discrimination by the sensor array.
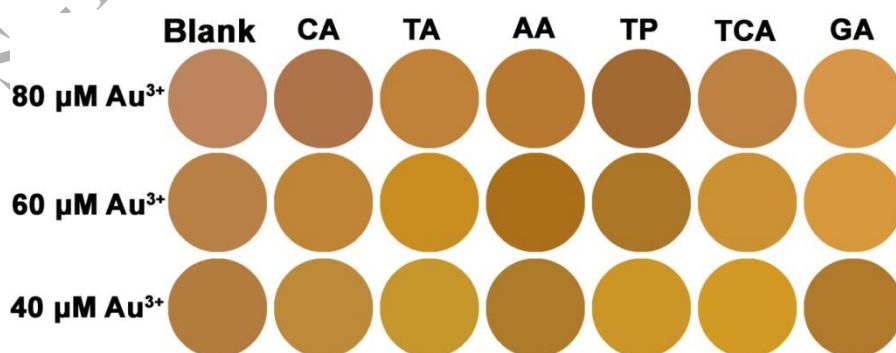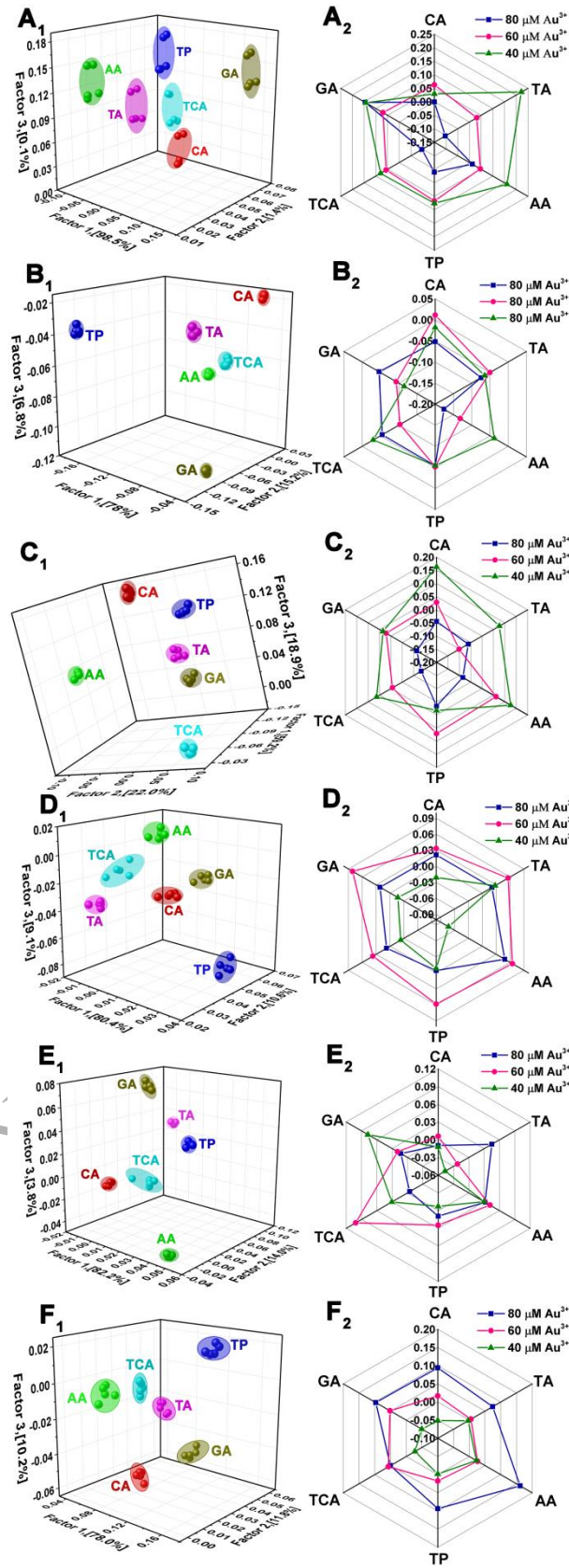


Figure 2 Photographs of Au@Ag NCs-based sensor array in the absence and presence of different antioxidants (all at 10 nM).

Figure 3 ($A_1$-$F_1$) Canonical score plots, and ($A_2$-$F_2$) radar plots derived from absorbance changes of the sensor array toward the six different antioxidants at ($A_1$,$A_2$) 200 nM, ($B_1$,$B_2$) 150 nM, ($C_1$,$C_2$) 100 nM, ($D_1$,$D_2$) 50 nM, ($E_1$,$E_2$) 20 nM, and ($F_1$,$F_2$) 10 nM.

## 3.4 Discrimination of mixtures of antioxidants

As far as we know, it has been a great challenge for sensor arrays to differentiate antioxidant mixtures with different molar ratios. We prepared different molar ratios (CA:TA=4:6, CA:TA:GA=4:3:3, AA:TP:TCA=3:3:4, CA:AA:TP:TCA=1:2:3:4, CA:TA:TP:TCA:GA=2:1:2:3, and CA:TA:AA:TP:TCA:GA=2:2:1:2:1:2, with 100 nM total antioxidant) of mixtures and discriminated them by the sensor array. As depicted in Fig. 4, these mixtures with different molar ratios were well-clustered into six clusters and separated from each other, clearly demonstrating the sensor array also possesses discriminative capacity for mixtures of antioxidants of different compositions.
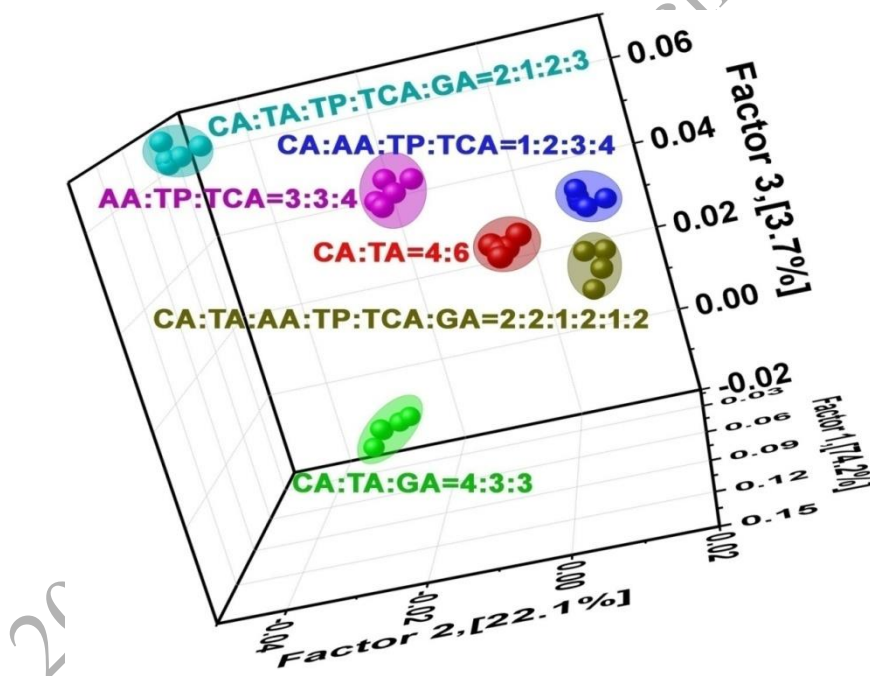


Figure 4 Canonical score plot for the colorimetric response patterns obtained against different molar ratios of the mixture of antioxidants.

## 3.5 Assay specificity

To assess the specificity of the as-fabricated assay, we selected three kinds of substances such as glutathione (GSH), histidine (His), and lysine (Lys) as interfering materials and investigated the effect of these species on the sensor array. As shown in Fig. 5A, although these interfering substances, each at 1μM, also produced obvious colorimetric responses, these responses were clearly distinct from those generated form the six target antioxidants at 100 nM. The result of the LDA analysis (Fig. 5B) satisfyingly reveals that the six target antioxidants can be discriminated effectively, and not any overlap occurs between the target antioxidants and the interfering substances. This result substantially suggested that the sensor array possessed a high degree of target specificity.
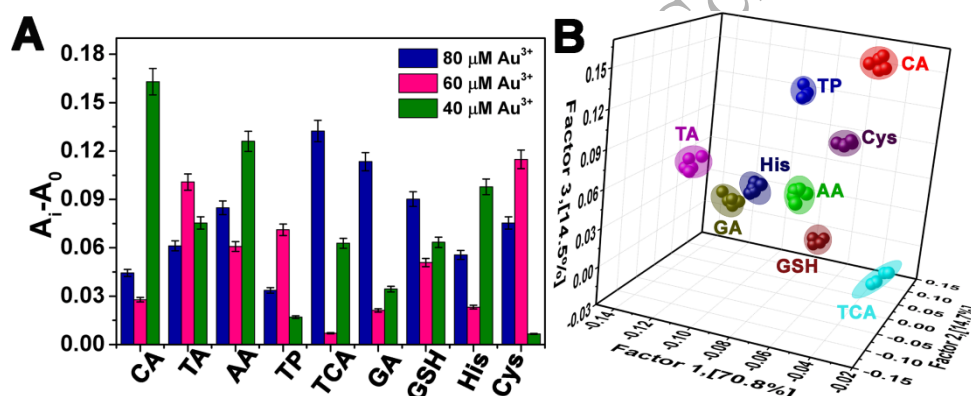


Figure 5 (A) Colorimetric response patterns (fingerprints) and (B) LDA score plots of the sensor array for identification of the six antioxidants (all at 100 nM) and the interfering species (all at 1 μM).

## 3.6 Application to real urine samples

To further assess the practicability of the sensor array, we investigated whether the sensor array can identify the six antioxidants in human urine sources. We spiked the urine samples collected from our laboratory staff with different antioxidants to obtain 20 nM of samples. The obtained absorbance data matrices were analysed by LDA and HCA. As shown in Fig. 6, it was found that there is not any

connection or overlapping for the seven clusters (urine itself and six antioxidants). These results demonstrate the potential applications of the developed sensor array for effective discrimination of multiple antioxidants in real urine samples.
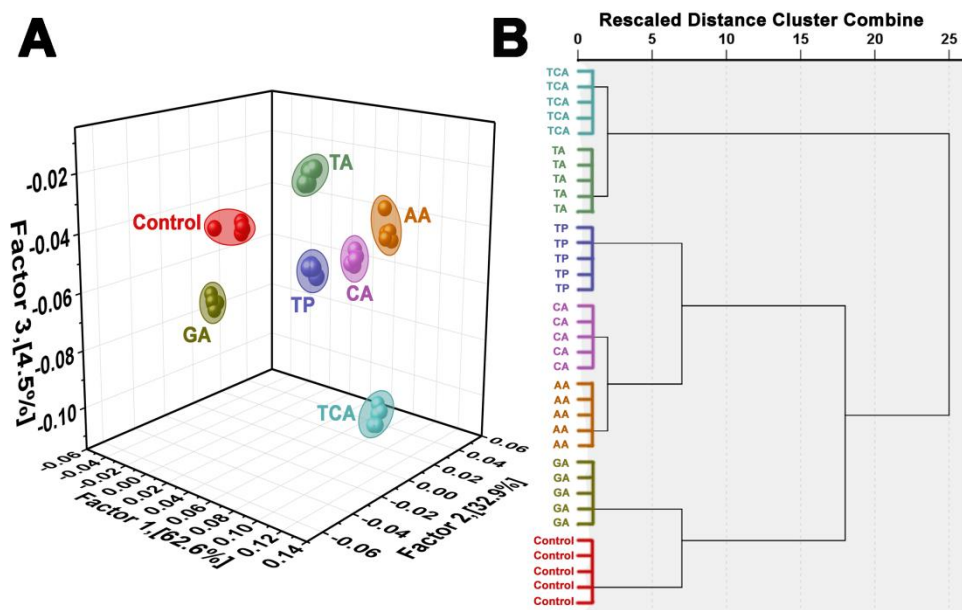


Figure 6.(A) Canonical score plot, and (B) HCA plot for the colorimetric response patterns obtained against 20 nM antioxidants in real urine samples.

# 4. Conclusion

In summary, a simple colorimetric sensor array consisting of three easy-to-prepare receptor units (40 μM Au$^{3+}$-Au@Ag NCs, 60 μM Au$^{3+}$-Au@Ag NCs, and 80 μM Au$^{3+}$-Au@Ag NCs) for discrimination of antioxidant species was developed. The sensor array shows that the differential interaction of target antioxidants with the sensing elements can inhibit the etching of Au@Ag NCs in varying degrees, which provided a characteristic fingerprint of the antioxidants. The sensor array demonstrated excellent ability to distinguish six antioxidants over a wide range of concentrations (10-200 nM) with 100% efficiency. Delightedly, the sensor array showed great anti-interferences and feasible potential for biofluids.

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# Appendix
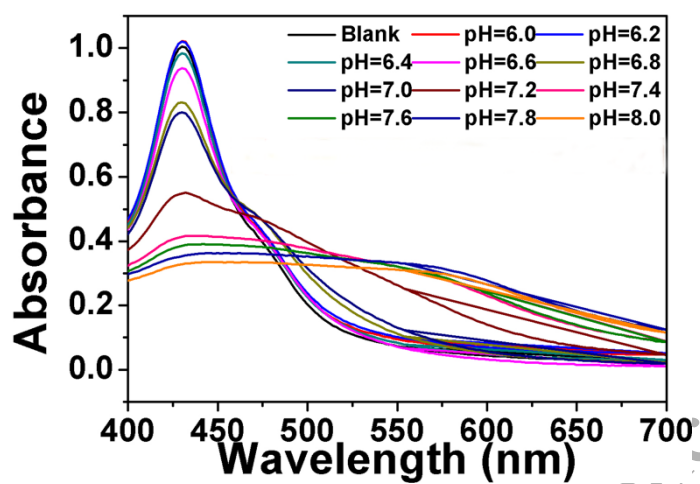


**Figure S1** UV-vis spectra of Au@Ag NCs in different pH of solutions in the presence of 40 μM HAuCl$_4$ and 20 nM AA.
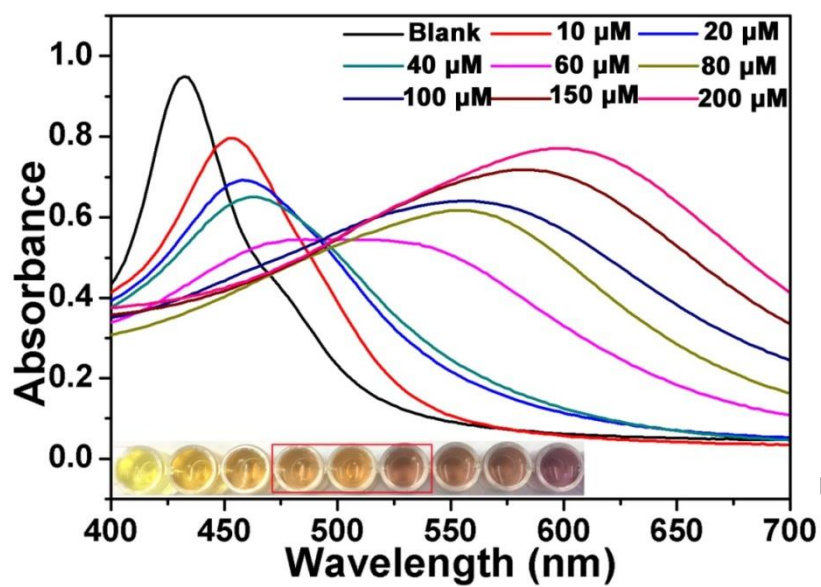
**Figure S2** UV-vis spectra of Au@Ag NCs in the presence of different concentrations of HAuCl₄.

**Table S1** The training matrix of the colorimetric response patterns against 6 antioxidants by using this sensor assay at the concentration of 200 nM.

| Antioxidants | $A_i - A_0$ 80 μM Au3+ | $A_i - A_0$ 60 μM Au3+ | $A_i - A_0$ 40 μM Au3+ |
|---|---|---|---|
| CA | -0.001 | 0.061 | 0.013 |
| CA | -0.004 | 0.063 | 0.013 |
| CA | -0.003 | 0.063 | 0.017 |
| CA | 0.001 | 0.065 | 0.051 |
| CA | 0.003 | 0.06 | 0.052 |
| TA | -0.064 | 0.017 | 0.116 |
| TA | -0.063 | 0.017 | 0.12 |
| TA | -0.06 | 0.023 | 0.12 |
| TA | -0.06 | 0.02 | 0.155 |
| TA | -0.063 | 0.017 | 0.155 |
| AA | 0.011 | 0.045 | 0.145 |
| AA | 0.011 | 0.044 | 0.145 |
| AA | 0.011 | 0.049 | 0.147 |
| AA | 0.011 | 0.047 | 0.182 |
| AA | 0.015 | 0.048 | 0.185 |
| TP | -0.039 | 0.066 | 0.06 |
| TP | -0.039 | 0.069 | 0.063 |
| TP | -0.04 | 0.072 | 0.059 |
| TP | -0.04 | 0.066 | 0.098 |
| TP | -0.038 | 0.064 | 0.1 |
| TCA | -0.098 | 0.054 | 0.065 |
| TCA | -0.097 | 0.056 | 0.065 |
| TCA | -0.094 | 0.06 | 0.063 |
| TCA | -0.096 | 0.057 | 0.104 |
| TCA | -0.098 | 0.053 | 0.102 |
| GA | 0.145 | 0.068 | 0.126 |
| GA | 0.144 | 0.07 | 0.129 |
| GA | 0.147 | 0.074 | 0.129 |
| GA | 0.146 | 0.069 | 0.166 |
| GA | 0.146 | 0.066 | 0.164 |

**Table S2** The training matrix of the colorimetric response patterns against 6 antioxidants by

using this sensor assay at the concentration of 150 nM.

| Antioxidants | Ai–A0 80 µM Au3+ | Ai–A0 60 µM Au3+ | Ai–A0 40 µM Au3+ |
|---|---|---|---|
| CA | -0.051 | 0.011 | -0.018 |
| CA | -0.052 | 0.011 | -0.017 |
| CA | -0.051 | 0.011 | -0.017 |
| CA | -0.052 | 0.013 | -0.017 |
| CA | -0.052 | 0.01 | -0.02 |
| TA | -0.074 | -0.051 | -0.065 |
| TA | -0.074 | -0.051 | -0.065 |
| TA | -0.074 | -0.048 | -0.064 |
| TA | -0.076 | -0.048 | -0.063 |
| TA | -0.075 | -0.051 | -0.065 |
| AA | -0.176 | -0.133 | -0.038 |
| AA | -0.174 | -0.131 | -0.038 |
| AA | -0.176 | -0.129 | -0.04 |
| AA | -0.177 | -0.131 | -0.034 |
| AA | -0.177 | -0.134 | -0.04 |
| TP | -0.054 | -0.052 | -0.055 |
| TP | -0.052 | -0.05 | -0.053 |
| TP | -0.056 | -0.051 | -0.056 |
| TP | -0.054 | -0.049 | -0.051 |
| TP | -0.052 | -0.054 | -0.055 |
| TCA | -0.055 | -0.105 | -0.032 |
| TCA | -0.053 | -0.102 | -0.03 |
| TCA | -0.054 | -0.102 | -0.03 |
| TCA | -0.056 | -0.105 | -0.027 |
| TCA | -0.056 | -0.105 | -0.032 |
| GA | -0.046 | -0.093 | -0.117 |
| GA | -0.047 | -0.091 | -0.115 |
| GA | -0.048 | -0.09 | -0.115 |
| GA | -0.045 | -0.097 | -0.114 |
| GA | -0.045 | -0.095 | -0.115 |

**Table S3** The training matrix of the colorimetric response patterns against 6 antioxidants by using this sensor assay at the concentration of 100 nM.

| Antioxidants | Ai–A0 80 μM Au3+ | Ai–A0 60 μM Au3+ | Ai–A0 40 μM Au3+ |
|---|---|---|---|
| CA | -0.044 | 0.028 | 0.158 |
| CA | -0.049 | 0.026 | 0.162 |
| CA | -0.043 | 0.028 | 0.169 |
| CA | -0.045 | 0.029 | 0.166 |
| CA | -0.041 | 0.028 | 0.16 |
| TA | -0.063 | -0.102 | 0.074 |
| TA | -0.061 | -0.099 | 0.079 |
| TA | -0.058 | -0.094 | 0.076 |
| TA | -0.063 | -0.101 | 0.078 |
| TA | -0.061 | -0.108 | 0.07 |
| AA | -0.084 | 0.06 | 0.125 |
| AA | -0.084 | 0.065 | 0.126 |
| AA | -0.087 | 0.067 | 0.132 |
| AA | -0.084 | 0.059 | 0.124 |
| AA | -0.085 | 0.053 | 0.123 |
| TP | -0.035 | 0.072 | -0.02 |
| TP | -0.035 | 0.069 | -0.02 |
| TP | -0.032 | 0.078 | -0.013 |
| TP | -0.029 | 0.07 | -0.01 |
| TP | -0.037 | 0.067 | -0.022 |
| TCA | -0.132 | -0.006 | 0.06 |
| TCA | -0.137 | -0.001 | 0.058 |
| TCA | -0.134 | -0.007 | 0.067 |
| TCA | -0.127 | -0.007 | 0.071 |
| TCA | -0.132 | -0.014 | 0.058 |
| GA | -0.115 | 0.022 | 0.033 |
| GA | -0.119 | 0.024 | 0.039 |
| GA | -0.112 | 0.02 | 0.03 |
| GA | -0.109 | 0.023 | 0.034 |
| GA | -0.112 | 0.017 | 0.036 |

**Table S4** The training matrix of the colorimetric response patterns against 6 antioxidants by using this sensor assay at the concentration of 50 nM.

| Antioxidants | Ai–A0 80 μM Au3+ | Ai–A0 60 μM Au3+ | Ai–A0 40 μM Au3+ |
|---|---|---|---|
| CA | 0.021 | 0.036 | -0.021 |
| CA | 0.02 | 0.034 | -0.022 |
| CA | 0.017 | 0.032 | -0.023 |
| CA | 0.023 | 0.032 | -0.019 |
| CA | 0.026 | 0.032 | -0.02 |
| TA | 0.008 | 0.042 | 0.015 |
| TA | 0.007 | 0.042 | 0.011 |
| TA | 0.006 | 0.038 | 0.013 |
| TA | 0.01 | 0.04 | 0.02 |
| TA | 0.013 | 0.039 | 0.016 |
| AA | 0.034 | 0.049 | -0.077 |
| AA | 0.033 | 0.046 | -0.081 |
| AA | 0.03 | 0.05 | -0.077 |
| AA | 0.033 | 0.052 | -0.071 |
| AA | 0.038 | 0.046 | -0.074 |
| TP | -0.015 | 0.043 | -0.018 |
| TP | -0.012 | 0.045 | -0.022 |
| TP | -0.017 | 0.038 | -0.022 |
| TP | -0.014 | 0.047 | -0.011 |
| TP | -0.012 | 0.039 | -0.015 |
| TCA | -0.003 | 0.024 | -0.031 |
| TCA | -0.003 | 0.024 | -0.034 |
| TCA | -0.005 | 0.025 | -0.032 |
| TCA | -0.003 | 0.024 | -0.029 |
| TCA | -0.003 | 0.019 | -0.029 |
| GA | 0.009 | 0.064 | -0.027 |
| GA | 0.011 | 0.064 | -0.027 |
| GA | 0.008 | 0.059 | -0.027 |
| GA | 0.01 | 0.065 | -0.023 |
| GA | 0.009 | 0.062 | -0.023 |

**Table S5** The training matrix of the colorimetric response patterns against 6 antioxidants by using this sensor assay at the concentration of 20 nM.

| Antioxidants | Ai–A0 80 μM Au3+ | Ai–A0 60 μM Au3+ | Ai–A0 40 μM Au3+ |
|---|---|---|---|
| CA | -0.008 | 0.007 | -0.01 |
| CA | -0.013 | 0.007 | -0.015 |
| CA | -0.009 | 0.004 | -0.013 |
| CA | -0.011 | 0.008 | -0.014 |
| CA | -0.008 | 0.004 | -0.009 |
| TA | 0.046 | -0.021 | -0.046 |
| TA | 0.045 | -0.022 | -0.047 |
| TA | 0.044 | -0.022 | -0.048 |
| TA | 0.045 | -0.021 | -0.048 |
| TA | 0.045 | -0.026 | -0.044 |
| AA | 0.033 | 0.042 | 0.03 |
| AA | 0.032 | 0.043 | 0.03 |
| AA | 0.03 | 0.043 | 0.032 |
| AA | 0.03 | 0.039 | 0.027 |
| AA | 0.034 | 0.04 | 0.03 |
| TP | 0.014 | 0.026 | -0.005 |
| TP | -0.003 | 0.028 | -0.009 |
| TP | 0.011 | 0.025 | -0.005 |
| TP | 0.011 | 0.025 | -0.011 |
| TP | 0.014 | 0.019 | -0.006 |
| TCA | -0.003 | 0.102 | 0.032 |
| TCA | -0.005 | 0.104 | 0.029 |
| TCA | -0.005 | 0.103 | 0.029 |
| TCA | -0.004 | 0.101 | 0.03 |
| TCA | -0.004 | 0.096 | 0.033 |
| GA | 0.014 | 0.02 | 0.078 |
| GA | 0.012 | 0.023 | 0.075 |
| GA | 0.016 | 0.018 | 0.075 |
| GA | 0.012 | 0.018 | 0.077 |
| GA | 0.012 | 0.018 | 0.082 |

**Table S6** The training matrix of the colorimetric response patterns against 6 antioxidants by using this sensor assay at the concentration of 10 nM.ice

| Antioxidants | Ai–A0 80 μM Au3+ | Ai–A0 60 μM Au3+ | Ai–A0 40 μM Au3+ |
|---|---|---|---|
| CA | 0.095 | 0.016 | -0.051 |
| CA | 0.094 | 0.016 | -0.049 |
| CA | 0.094 | 0.014 | -0.05 |
| CA | 0.094 | 0.019 | -0.057 |
| CA | 0.093 | 0.018 | -0.05 |
| TA | 0.074 | 0.005 | -0.004 |
| TA | 0.075 | 0.005 | 0.001 |
| TA | 0.076 | 0.003 | 0 |
| TA | 0.072 | 0.002 | -0.009 |
| TA | 0.074 | 0.011 | -0.006 |
| AA | 0.16 | 0.022 | 0.022 |
| AA | 0.163 | 0.027 | 0.026 |
| AA | 0.165 | 0.028 | 0.024 |
| AA | 0.159 | 0.026 | 0.02 |
| AA | 0.16 | 0.028 | 0.02 |
| TP | 0.094 | 0.017 | -0.003 |
| TP | 0.095 | 0.017 | 0.001 |
| TP | 0.097 | 0.015 | 0.003 |
| TP | 0.09 | 0.019 | -0.007 |
| TP | 0.093 | 0.019 | -0.002 |
| TCA | 0.05 | 0.054 | -0.027 |
| TCA | 0.048 | 0.055 | -0.024 |
| TCA | 0.05 | 0.056 | -0.024 |
| TCA | 0.051 | 0.058 | -0.032 |
| TCA | 0.052 | 0.059 | -0.031 |
| GA | 0.097 | 0.05 | -0.05 |
| GA | 0.1 | 0.053 | -0.049 |
| GA | 0.099 | 0.054 | -0.045 |
| GA | 0.094 | 0.048 | -0.05 |
| GA | 0.094 | 0.052 | -0.053 |

**Table S7** The training matrix of the colorimetric response patterns against the mixture of

2/3/4/5 and 6 kinds of antioxidants at 100 nM by using this sensor assay.

| Antioxidants | Ai–A0 80 μM Au3+ | Ai–A0 60 μM Au3+ | Ai–A0 40 μM Au3+ |
|---|---|---|---|
| CA:TA=4:6 | 0.131 | 0.001 | 0.038 |
| CA:TA=4:6 | 0.129 | 0 | 0.039 |
| CA:TA=4:6 | 0.13 | 0.004 | 0.04 |
| CA:TA=4:6 | 0.13 | 0.002 | 0.038 |
| CA:TA=4:6 | 0.133 | 0.001 | 0.037 |
| AA:TP:TCA=3:3:4 | 0.025 | -0.032 | -0.011 |
| AA:TP:TCA=3:3:4 | 0.022 | -0.032 | -0.012 |
| AA:TP:TCA=3:3:4 | 0.022 | -0.027 | -0.01 |
| AA:TP:TCA=3:3:4 | 0.021 | -0.029 | -0.011 |
| AA:TP:TCA=3:3:4 | 0.025 | -0.034 | -0.015 |
| CA:TA:GA=4:3:3 | 0.039 | 0.01 | 0.033 |
| CA:TA:GA=4:3:3 | 0.039 | 0.01 | 0.034 |
| CA:TA:GA=4:3:3 | 0.035 | 0.014 | 0.032 |
| CA:TA:GA=4:3:3 | 0.04 | 0.01 | 0.036 |
| CA:TA:GA=4:3:3 | 0.043 | 0.011 | 0.032 |
| CA:AA:TP:TCA=1:2:3:4 | 0.067 | -0.047 | 0.064 |
| CA:AA:TP:TCA=1:2:3:4 | 0.067 | -0.047 | 0.068 |
| CA:AA:TP:TCA=1:2:3:4 | 0.062 | -0.042 | 0.065 |
| CA:AA:TP:TCA=1:2:3:4 | 0.066 | -0.045 | 0.064 |
| CA:AA:TP:TCA=1:2:3:4 | 0.067 | -0.047 | 0.063 |
| CA:TA:TP:TCA:GA=2:1:2:3:2 | 0.071 | -0.018 | 0.046 |
| CA:TA:TP:TCA:GA=2:1:2:3:2 | 0.069 | -0.016 | 0.05 |
| CA:TA:TP:TCA:GA=2:1:2:3:2 | 0.07 | -0.012 | 0.049 |
| CA:TA:TP:TCA:GA=2:1:2:3:2 | 0.071 | -0.015 | 0.047 |
| CA:TA:TP:TCA:GA=2:1:2:3:2 | 0.075 | -0.017 | 0.045 |
| CA:TA:AA:TP:TCA:GA=2:2:1:2:1:2 | 0.05 | 0.011 | 0.019 |
| CA:TA:AA:TP:TCA:GA=2:2:1:2:1:2 | 0.047 | 0.009 | 0.023 |
| CA:TA:AA:TP:TCA:GA=2:2:1:2:1:2 | 0.044 | 0.012 | 0.022 |
| CA:TA:AA:TP:TCA:GA=2:2:1:2:1:2 | 0.051 | 0.011 | 0.019 |

| CA:TA:AA:TP:TCA: GA=2:2:1:2:1:2 | 0.052 | 0.009 | 0.015 |

**Table S8** The training matrix of the colorimetric response patterns against the 6 antioxidants at 20 nM in urine samples by using this sensor assay.

| Antioxidants | $A_i - A_0$ 80 μM $Au^{3+}$ | $A_i - A_0$ 60 μM $Au^{3+}$ | $A_i - A_0$ 40 μM $Au^{3+}$ |
|---|---|---|---|
| Serum | 0.02 | -0.02 | -0.039 |
| Serum | -0.001 | -0.024 | -0.04 |
| Serum | 0.021 | -0.022 | -0.04 |
| Serum | 0.017 | -0.019 | -0.037 |
| Serum | 0.022 | -0.022 | -0.038 |
| CA | 0.072 | 0.019 | -0.05 |
| CA | 0.075 | 0.015 | -0.05 |
| CA | 0.07 | 0.016 | -0.052 |
| CA | 0.069 | 0.02 | -0.048 |
| CA | 0.071 | 0.018 | -0.053 |
| TA | 0.118 | -0.052 | -0.037 |
| TA | 0.119 | -0.053 | -0.037 |
| TA | 0.121 | -0.05 | -0.04 |
| TA | 0.117 | -0.05 | -0.037 |
| TA | 0.121 | -0.052 | -0.037 |
| AA | 0.073 | 0.043 | -0.045 |
| AA | 0.074 | 0.038 | -0.036 |
| AA | 0.075 | 0.043 | -0.048 |
| AA | 0.069 | 0.044 | -0.046 |
| AA | 0.073 | 0.04 | -0.047 |
| TP | 0.079 | 0.031 | -0.108 |
| TP | 0.08 | 0.027 | -0.111 |
| TP | 0.081 | 0.032 | -0.107 |
| TP | 0.077 | 0.033 | -0.105 |
| TP | 0.082 | 0.028 | -0.111 |
| TCA | 0.124 | -0.057 | -0.008 |
| TCA | 0.124 | -0.058 | -0.011 |
| TCA | 0.126 | -0.056 | -0.007 |
| TCA | 0.121 | -0.055 | -0.008 |
| TCA | 0.123 | -0.059 | -0.011 |
| GA | -0.037 | -0.001 | -0.074 |
| GA | -0.036 | -0.002 | -0.073 |
| GA | -0.035 | 0.001 | -0.074 |
| GA | -0.04 | 0 | -0.071 |
| GA | -0.034 | -0.002 | -0.076 |