Supporting Information

Particle-in-a-frame gold nanomaterials with interior nanogap-based sensor array for versatile analyte detection

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Experimental Section

Chemicals and Materials. $HAuCl_4 \cdot 4H_2O$ was obtained from Sinopharm Chemical Reagent Company (Beijing, China). Melatonin (MT), L-cysteine (L-Cys), uric acid (UA), glutathione (GSH), 3,3′,5,5′-tetramethylbenzidine (TMB), potassium iodide (KI), and silver nitrate ($AgNO_3$) were purchased from Aladdin Industrial Corporation (Shanghai, China). Lysozyme (Lys), human serum albumin (HSA), horseradish peroxidase (HRP), bovine albumin (BSA), trypsin (Try), haemoglobin (Hem), immunoglobulin G (IgG), hydrogen peroxide (H_2O_2), and sodium citrate were acquired from Innochem Chemical Reagent Company (Beijing, China). PVP (MW = 55,000), sodium borohydride ($NaBH_4$), and L-ascorbic acid (AA) were obtained from Sigma (St. Louis, MO, USA). Acetic acid (HAc) and sodium acetate (NaAc) were acquired from Damao Chemical Reagent Factory (Tianjin, China). Sodium chloride was purchased from Beijing Chemical Works (Beijing, China). Ultrapure water was prepared with a Milli-Q water purification system (Millipore, Milford, MA, USA).

Instrumentation. Transmission electron microscopy (TEM) images were obtained using a JEOL 2010 transmission electron microscope. Circular dichroism (CD) spectra were obtained on a J-810 spectropolarimeter (JASCO). Absorption spectra were acquired using a microplate reader (Varioskan LUX Filter, Thermo Fisher Scientific, Waltham, USA). The isothermal titration calorimetry (ITC) data were obtained by an ITC 200 (Malvern Instruments Ltd, UK). The linear discriminant analysis (LDA) diagram was processed by SYSTAT 13.0 software. Hierarchical cluster analysis (HCA) was obtained from IBM SPSS Statistics 20.0.

Synthesis of nanomaterials. Preparation of Ag nanoprisms [1]: 600 μL of 5 mM $AgNO_3$ was mixed with 25.5 mL of deionized water in a conical bottle. Sodium citrate (1.8 mL, 30 mM) and PVP (1.8 mL, 5 $mg \cdot mL^{-1}$) were added and stirred vigorously at room temperature. After a few minutes, hydrogen peroxide (60 μL) and sodium borohydride (300 μL, 100 mM) were quickly added. After stirring for 30 minutes, Ag nanoprisms were synthesized.

Synthesis of Au PIAF [1]: PVP (4 mL, 10 $mg \cdot mL^{-1}$) and deionized water (3.2 mL) were added into a conical bottle. $HAuCl_4 \cdot 4H_2O$ (200 μL, 10 mM), KI (40 μL, 50 mM) and AA (200 μL, 100 mM) were added sequentially and the solution was mixed gently. The Ag nanoprism seed solution (2 mL) was quickly added and the solution was shaken evenly. The solution was left at 0 °C for 30 minutes.

Discrimination of multiple substances. Forty microlitres of Au PIAF was incubated with 50 μL of protein for 30 minutes, and then 40 μL of TMB (10 mM), 30 μL of hydrogen peroxide (3 M) and 340 μL of buffer solution (pH = 4, 0.2 M) were added to the system. The system was incubated at 37 °C for 40 minutes. The method for detecting antioxidants and cells was the same as that for proteins, except for changes in substances. The methods of cell treatment were described in previous literature.[2] The concentration of each cell was diluted to the same concentration (approximately 85000/mL). We used the serum solution of healthy people, which was centrifuged at 14000 r/min to remove insoluble matter and impurities. After that, the serum was diluted twice with ultrapure water.

Results and Discussion

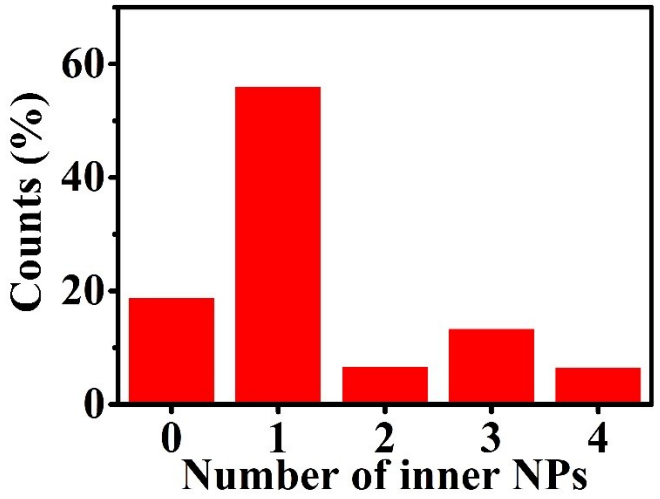

Fig. S1 The distribution of Au PIAF containing the number of inner NPs obtained from the TEM diagram

Table S1 Basic properties and manufacturers of proteins

Protein	MW (kDa)	pI	Metal
Hemoglobin (Hem)	64.5	6.8	Yes
Trypsin (Try)	24	10.5	No
Horseradish peroxidase (HRP)	~40	3.9	Yes
Human serum albumin (HSA)	69.4	5.2	No
Lysozyme (Lys)	14.4	11.0	No
Bovine serum albumin (BSA)	66.3	4.8	No
Immunoglobulin G (IgG)	150	7.5	No

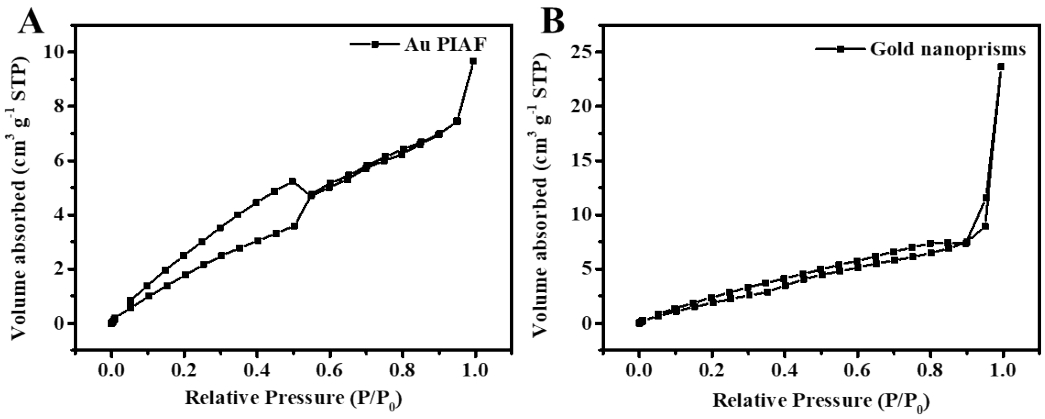

Fig. S2 Nitrogen adsorption/desorption isotherms of Au PIAF and gold nanoprisms. The specific surface area of Au PIAF is 11.333 m^2 g^{-1}, the specific surface area of gold nanoprisms is 10.688 m^2 g^{-1}.

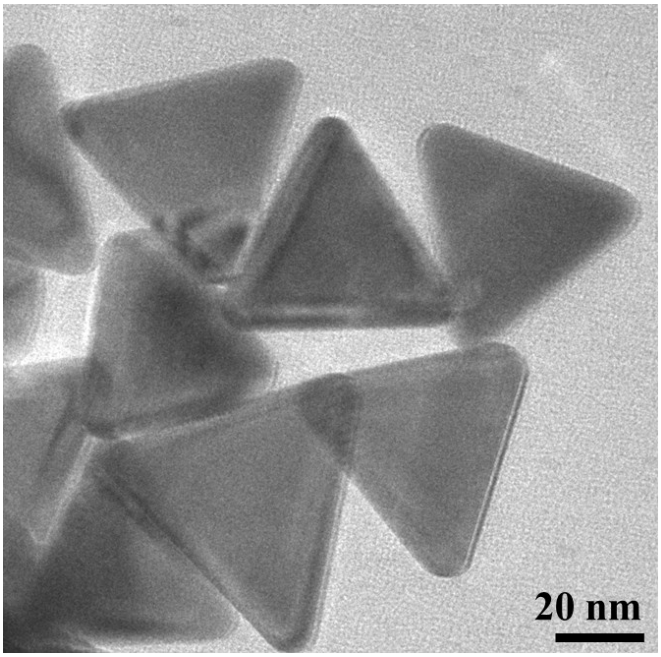

Fig. S3 TEM images of gold nanoprisms. Gold nanoprisms was prepared using previously reported methods.[3]

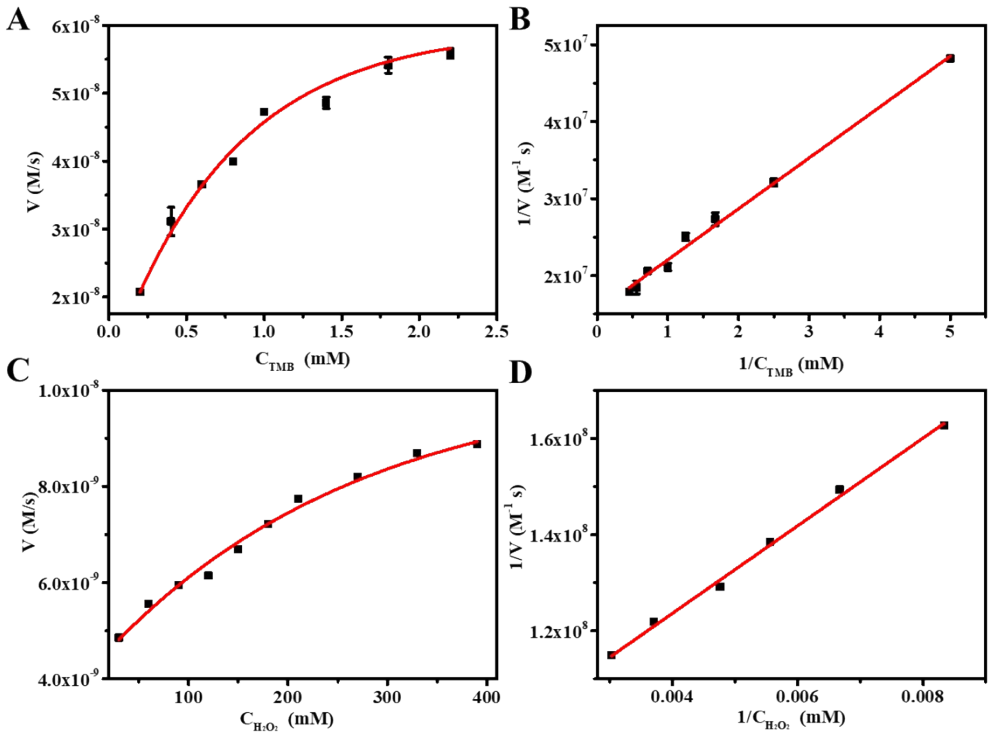

Fig. S4 Steady-state kinetic assays of the Au PIAF for the oxidation of TMB by H_2O_2. The kinetic data were obtained by varying one substrate concentrations while keeping the other substrate concentration constant (A, C). The Lineweaver-Burk plots (B, D) of the double reciprocal of the Michaelis-Menten equations.

Table **2** Comparison of the Kinetic Parameters of various enzyme

Catalyst	Substrate	K_m [mM]	V_{max} [10^{-8} M S^{-1}]	References
PBNPs	TMB	0.34	21.60	4
	H_2O_2	14.70	11.50	
Fe-MOF	TMB	2.60	5.60	5
	H_2O_2	1.30	2.50	
HRP	TMB	0.43	10.00	6
	H_2O_2	0.07	0.56	
Au PIAF	TMB	0.42	6.47	This work
	H_2O_2	10.43	1.14	

The maximum initial velocity (V_{max}) and Michaelis-Menten constant (K_m) were estimated by Lineweaver-Burk plot, $1/V = K_m/V_{max}[S] + 1/V_{max}$; where V was the initial reaction rate and [S] was the concentration of the substrate.

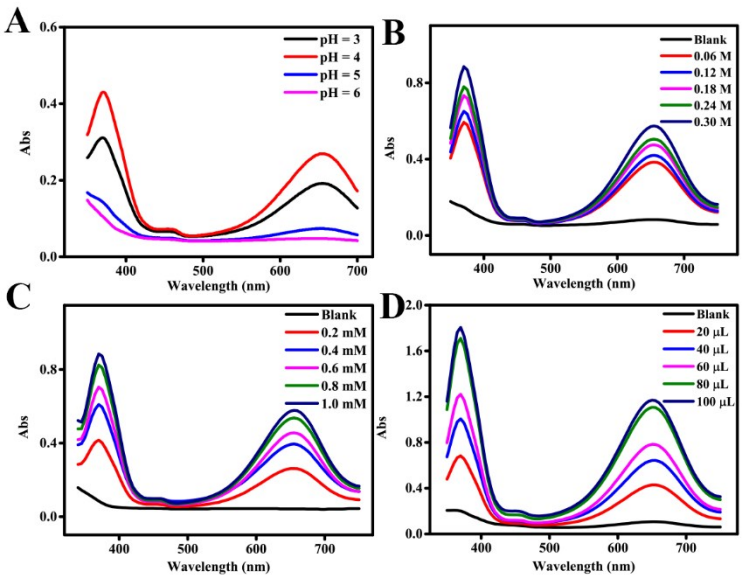

Fig. S5 UV-vis spectra under different conditions A) pH; B) H_2O_2; C) TMB; D) Au PIAF.

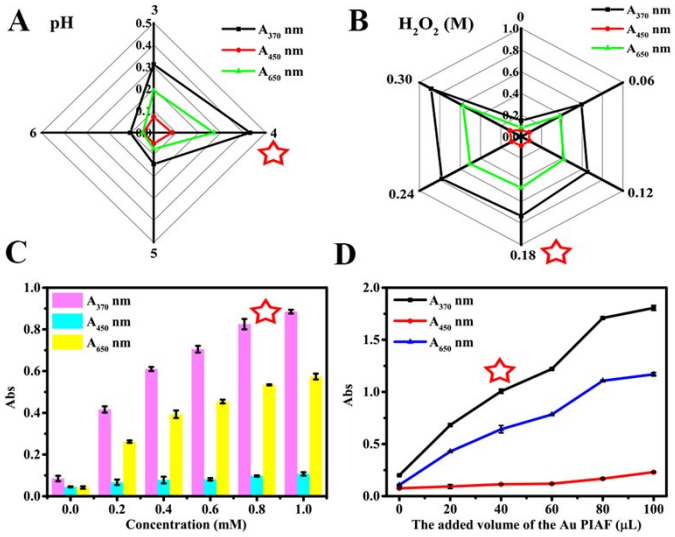

Fig. S6 Changes in absorbance values under different conditions A) pH; B) H_2O_2; C) TMB; D) Au PIAF.

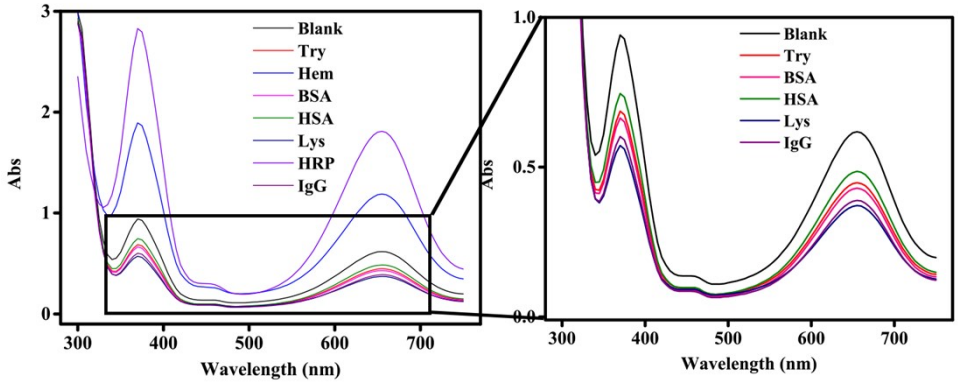

Fig. S7 The UV-vis spectra of array in presence of different proteins.

Table S3 Identification of unknown protein samples (50 nM) using the colorimetric sensor array in aqueous solution

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.184	0.188	0.301	Try	Try
2	0.267	0.179	0.256	BSA	BSA
3	0.401	0.161	0.384	Lys	Lys
4	-2.275	-0.866	-0.811	HRP	HRP
5	0.314	0.107	0.251	IgG	IgG
6	0.239	0.107	0.241	Try	Try
7	-0.864	-1.884	-2.301	Hem	Hem
8	0.176	0.134	0.230	HSA	HSA
9	0.401	0.214	0.424	Lys	Lys
10	0.257	0.125	0.244	BSA	BSA
11	0.247	0.121	0.239	BSA	HSA
12	-2.187	-0.795	-0.774	HRP	HRP
13	0.305	0.161	0.244	IgG	IgG
14	-0.829	-1.688	-2.226	Hem	Hem

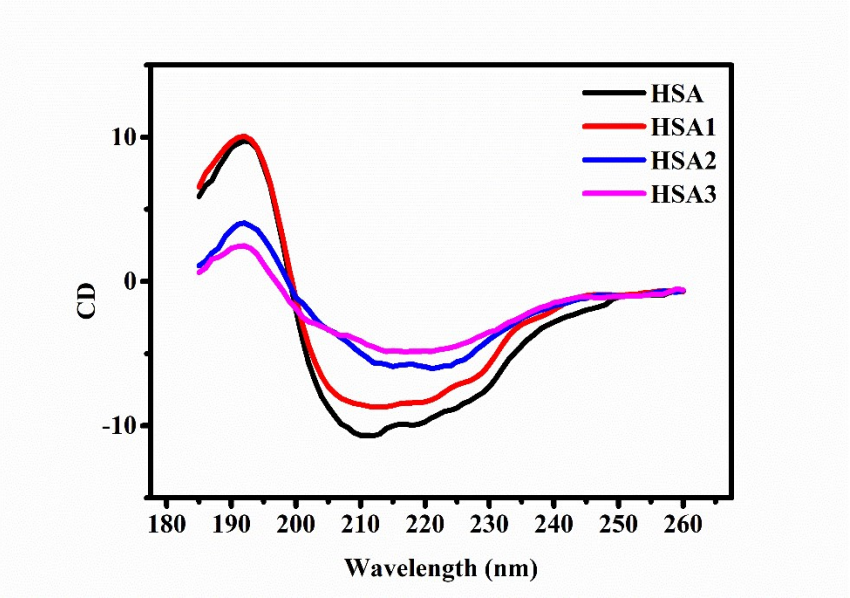

Fig. S8 Circular dichroism spectra of HSA protein: 1) heated at 60 °C for 10 min, 2) heated at 80 °C for 10 min; 3) heated at 80 °C for 20 min.

Table S4 Secondary structures of native proteins and thermally denatured proteins: 1) heated at 60 °C for 10 min, 2) heated at 80 °C for 10 min; 3) heated at 80 °C for 20 min.)

	HSA	HSA1	HSA2	HSA3
α %	30.9	28.7	21.5	19.0
β %	21.1	23.8	24.8	27.4
Turn %	18.0	18.3	19.4	20.1
Random %	29.2	31.9	42.6	45.2

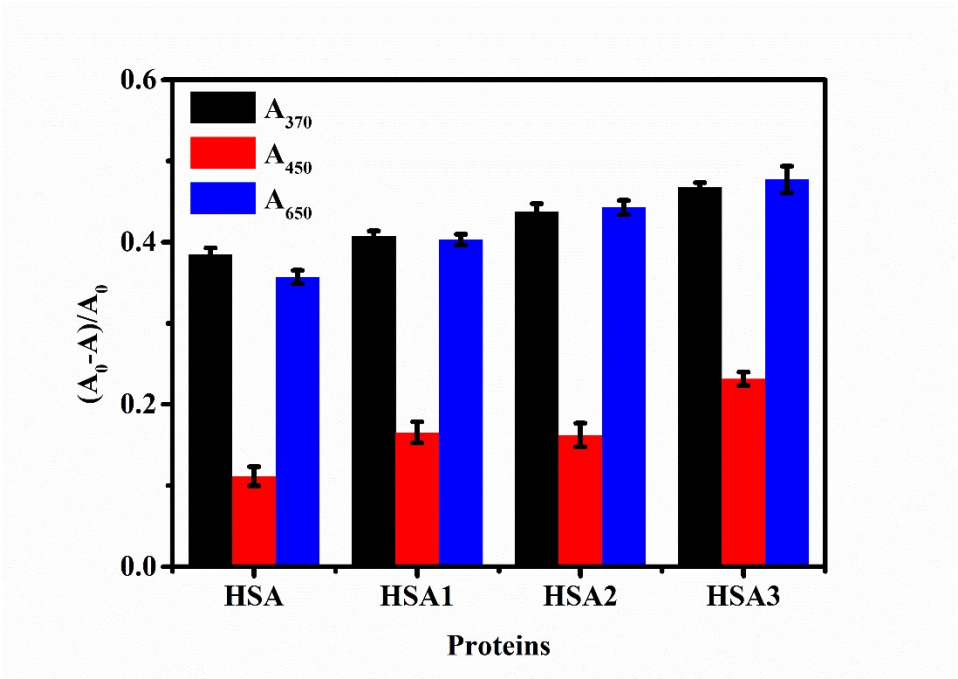

Fig. S9 Absorbance response for native proteins and thermally denatured proteins

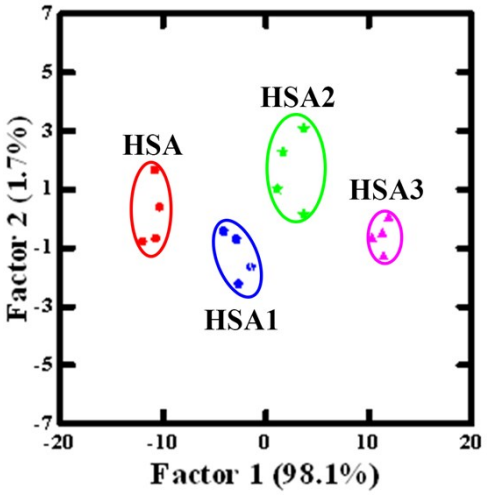

Fig. S10 LDA for thermally denatured proteins.

Table S5 Identification of unknown protein samples (native and thermally denatured proteins) using the sensor array

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.380	0.122	0.344	HSA	HSA
2	0.476	0.243	0.523	HSA3	HSA3
3	0.430	0.149	0.427	HSA2	HSA2
4	0.418	0.162	0.387	HSA1	HSA1
5	0.440	0.182	0.459	HSA2	HSA2
6	0.407	0.176	0.378	HSA1	HSA1
7	0.475	0.223	0.505	HSA3	HSA3
8	0.398	0.115	0.333	HSA	HSA

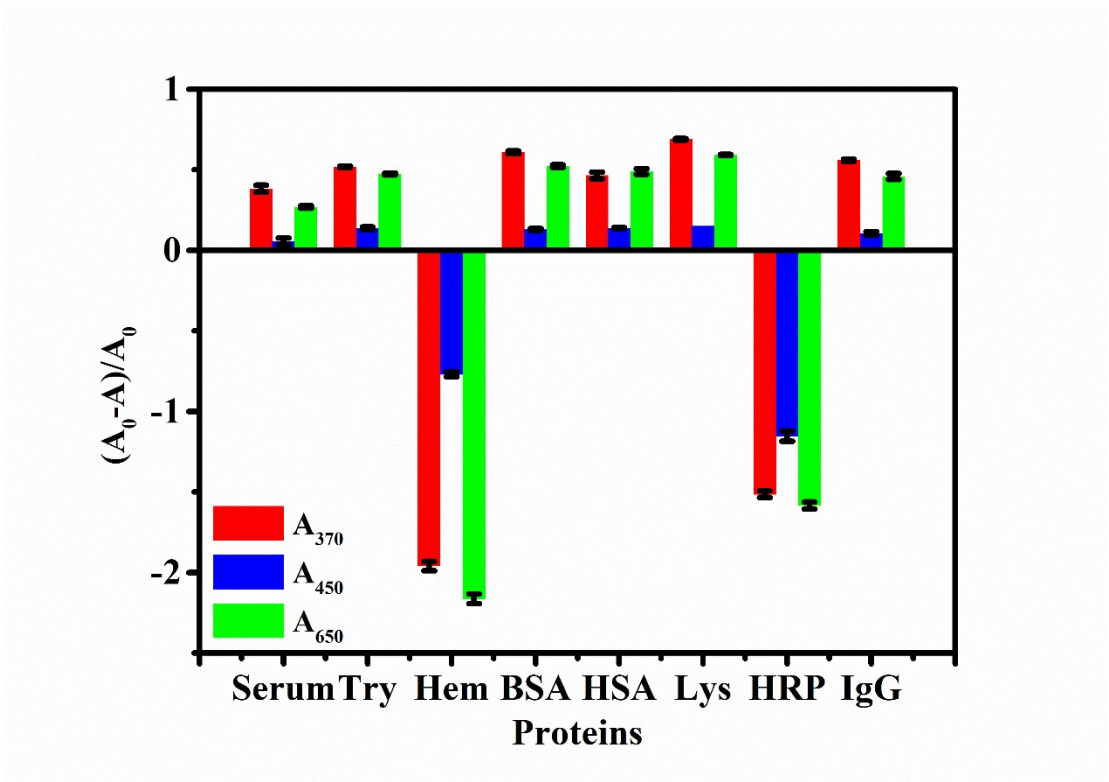

Fig. S11 Fingerprints of protein samples in serum based on the colorimetric sensor array

Table S6 Identification of unknown protein samples in serum using the sensor array

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.368	0.036	0.256	Serum	Serum
2	0.353	0.027	0.266	Serum	Serum
3	0.520	0.116	0.485	Try	Try
4	-1.450	-1.179	-1.532	HRP	HRP
5	0.554	0.107	0.431	IgG	IgG
6	0.441	0.143	0.522	HSA	HSA
7	-1.923	-0.759	-2.121	Hem	Hem
8	0.595	0.116	0.552	BSA	BSA
9	0.454	0.134	0.517	HSA	HSA
10	-1.911	-0.795	-2.114	Hem	Hem
11	0.679	0.143	0.586	Lys	Lys
12	0.690	0.143	0.584	Lys	Lys
13	0.521	0.116	0.470	Try	Try
14	-1.478	-1.196	-1.557	HRP	HRP
15	0.614	0.116	0.532	BSA	BSA
16	0.563	0.098	0.433	IgG	IgG

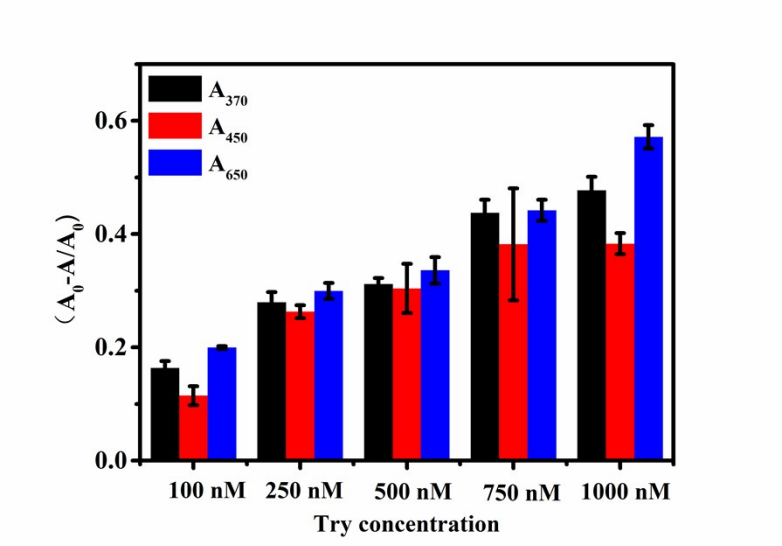

Fig. S12 Fingerprints of Try at various concentrations based on the colorimetric sensor array (Here, A_0 was the absorbance value of the serum sample, and A was the absorbance value when the target was present)

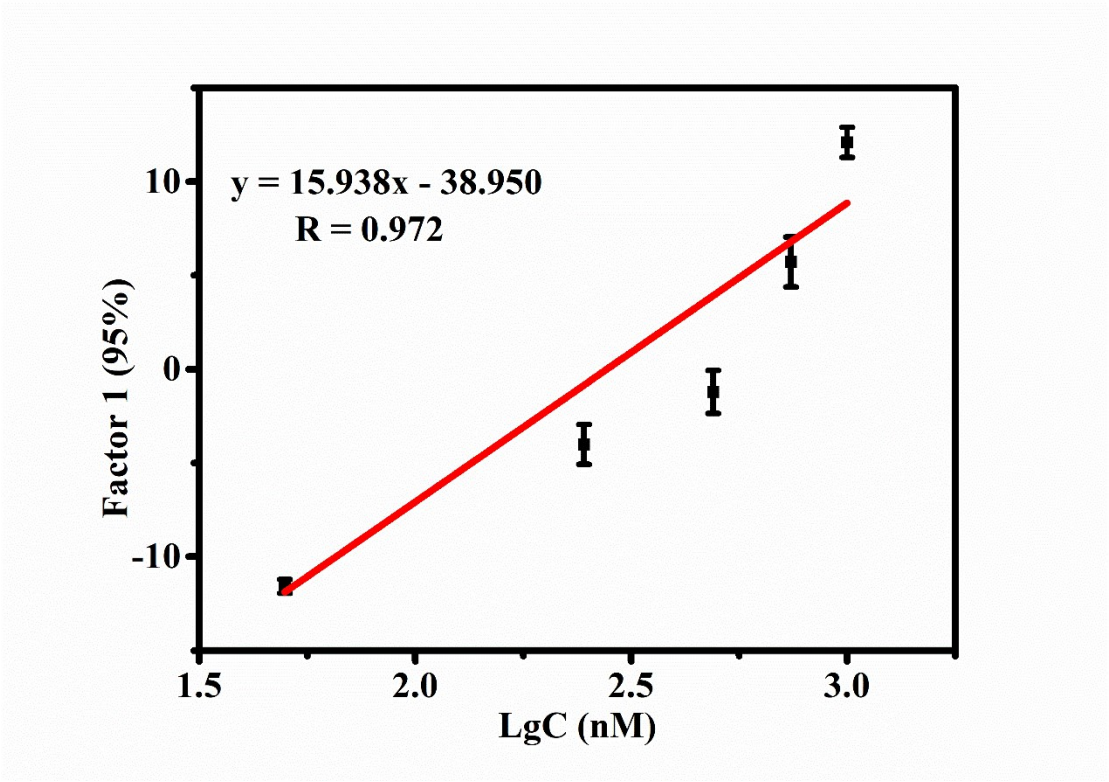

Fig. S13 Plot of the discriminant Factor (1) versus the logarithm of the Try concentration.

Table S7 Identification of unknown protein samples (Try at different concentration) using the colorimetric sensor array

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.153	0.143	0.193	100 nM	100 nM
2	0.397	0.423	0.416	750 nM	750 nM
3	0.470	0.439	0.541	1000 nM	1000 nM
4	0.258	0.295	0.284	250 nM	250 nM
5	0.324	0.359	0.327	500 nM	500 nM
6	0.323	0.327	0.308	500 nM	500 nM
7	0.411	0.431	0.431	750 nM	750 nM
8	0.139	0.079	0.180	100 nM	100 nM
9	0.252	0.279	0.280	250 nM	250 nM
10	0.478	0.399	0.509	1000 nM	1000 nM

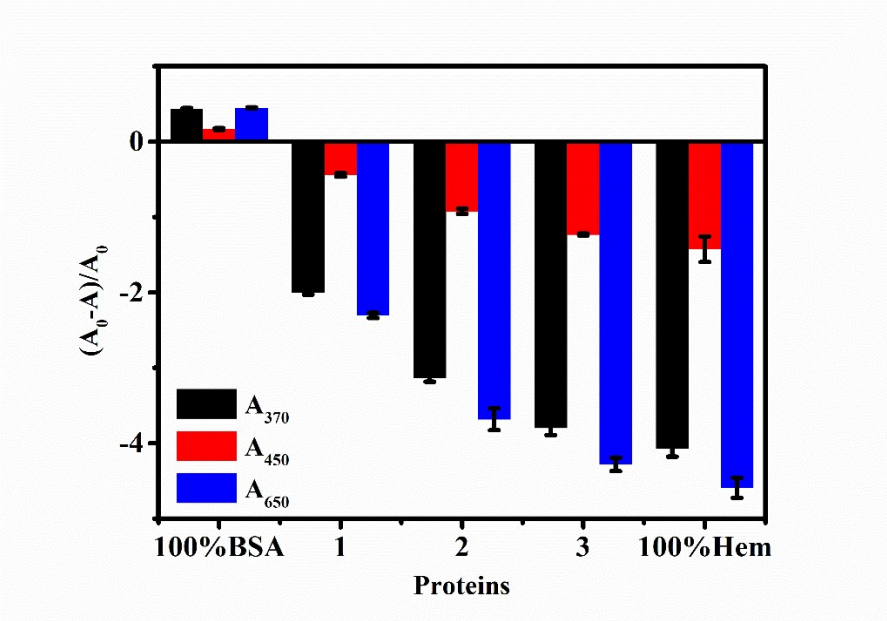

Fig. S14 Absorbance response for discrimination mixtures of BSA and Hem at different molar ratios (total protein concentration: 400 nM) (Here, A_0 was the absorbance value of the serum sample, and A was the absorbance value when the target was present).

Table S8 Identification of unknown protein samples at different molar ratios using the sensor array

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.403	0.175	0.427	100% BSA	100% BSA
2	-3.864	-1.230	-4.376	25% BSA+75% Hem	25% BSA+75% Hem
3	-4.099	-1.548	-4.627	100% Hem	100% Hem
4	-3.147	-0.889	-3.590	50% BSA+50% Hem	50% BSA+50% Hem
5	-1.917	-0.437	-2.393	75% BSA+25% Hem	75% BSA+25% Hem
6	-1.917	-0.413	-2.322	75% BSA+25% Hem	75% BSA+25% Hem
7	-3.762	-1.238	-4.260	25% BSA+75% Hem	25% BSA+75% Hem
8	-4.030	-1.357	-4.545	100% Hem	100% Hem
9	0.405	0.183	0.395	100% BSA	100% BSA
10	-3.235	-0.944	-3.692	50% BSA+50% Hem	50% BSA+50% Hem

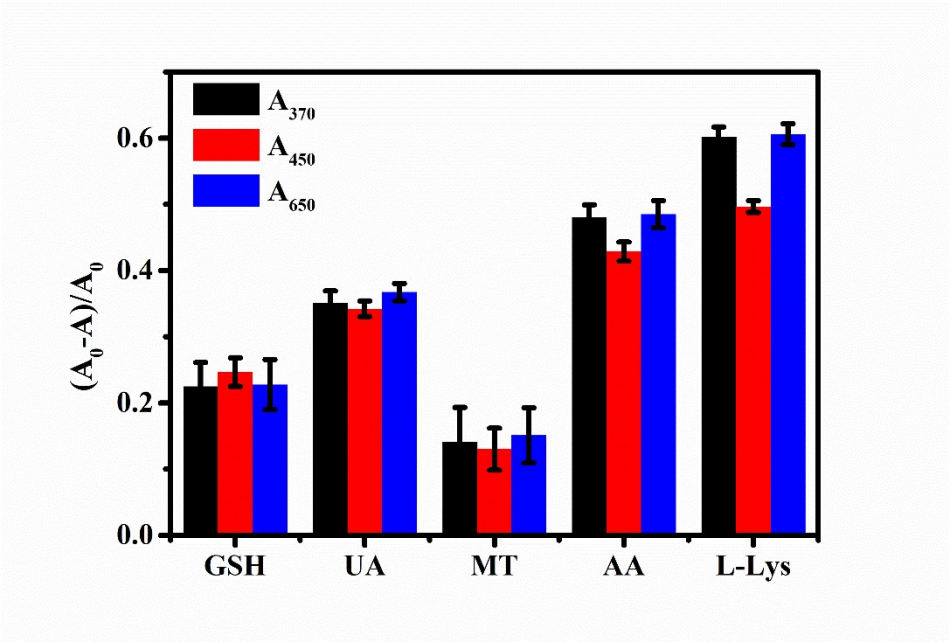

Fig. S15 Absorbance response for antioxidant samples.

Table S9 Identification of unknown antioxidant samples using the sensor array

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.290	0.229	0.295	GSH	GSH
2	0.564	0.486	0.572	L-Lys	L-Lys
3	0.489	0.438	0.492	AA	AA
4	0.328	0.326	0.334	UA	UA
5	0.331	0.326	0.336	UA	UA
6	0.095	0.090	0.244	MT	MT
7	0.495	0.431	0.498	AA	AA
8	0.278	0.222	0.283	GSH	GSH
9	0.112	0.083	0.231	MT	MT
10	0.572	0.493	0.576	L-Lys	L-Lys

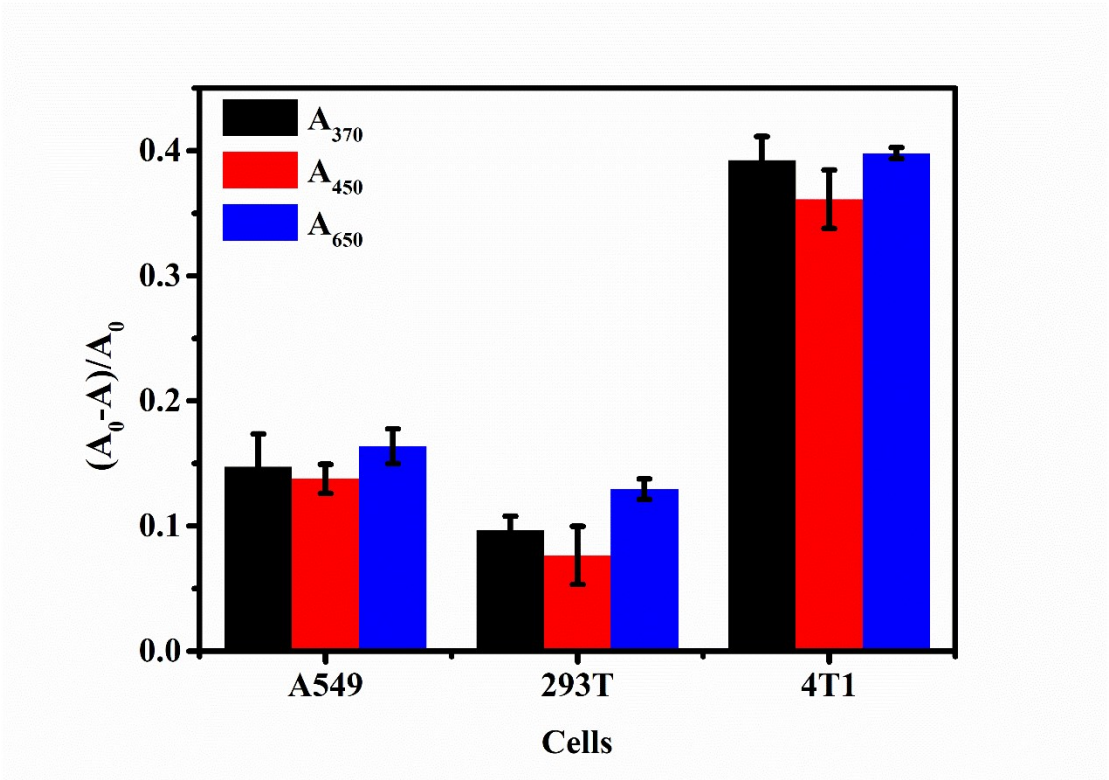

Fig. S16 Absorbance response for cell.

Table S10 Identification of unknown cell samples using the sensor array

Samples	A_{370}	A_{450}	A_{650}	Identification	Verification
1	0.174	0.147	0.167	A549	A549
2	0.417	0.392	0.414	4T1	4T1
3	0.100	0.086	0.077	293T	293T
4	0.398	0.380	0.415	4T1	4T1
5	0.153	0.184	0.182	A549	A549

References

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