Supplementary Information
for
Using Metal Nanoparticles as a Visual Sensor for the Discrimination of Proteins

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Experimental Section

1. The basic properties of ten proteins

Table S1. Basic Properties of proteins

Protein	MW (kDa)	pI
Human serum albumin (HSA)	69.4	5.2
Papain (Pap)	23.0	9.6
Catalase from bovine liver	200-340	8.3
Trypsin (Try) 1:250	24	10.5
Lysozyme (Lys)	14.4	9.6~11
Hemoglobin (Hb)	64.5	6.8
egg Albumin (EA)	44-45	4.6
Mucins from pig stomach	2.098	9.6-11
Human IgG	150-160	7.5-7.8
γ-Globulins from bovine blood	43	6.85-7.5

2. Preparation of one-dimensional polyacrylamide gel electrophoresis (1-D

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PAGE) and metal NPs-based FL imaging

The nondenaturing 1-D PAGE was performed in a vertical discontinuous gel system, consisting of separating (7.5%, m/v) and stacking (4.0%, m/v) gels. The average diameter of the gel pore is about 5 nm. Gel stock solution (30%, w/v) contained 29.2 g of acrylamide and 0.8 g of Bis, which were dissolved in 100 mL deionized water using ultrasonic instrument and then filtrated. The separating gel solution (7.5%, m/v) was prepared by mixing 4 mL of gel stock solution, 4 mL of Tris-HCl (1.5 M, pH 8.80), 8 mL of deionized water, 150 μL of $(NH_4)_2S_2O_8$ (10%, w/v) and 15 μL of TEMED. To prepare the stacking one (4.0%, m/v), 0.7 mL of gel stock solution was mixed with 1.25 mL of Tris-HCl (0.5 M, pH 6.80), 3 mL of deionized water, 40 μL of $(NH_4)_2S_2O_8$ (10%, w/v) and 4 μL of TEMED. Here the solution of $(NH_4)_2S_2O_8$ (10%, w/v) was prepared daily to ensure the stability. The electrophoresis buffer was prepared by dissolving 1.5 g of Tris and 7.2 g of glycine in 500 mL of deionized water, adjusting the pH to 8.30. The protein mixture (15 $mg \cdot mL^{-1}$, 400 μL) consisting of HSA, Hb and catalase, was mixed with 200 μL of glycerol (20%, v/v) and 200 μL of deionized water; the loading volume for each channel was 40 μL. The voltage was set at 120 V for 30 min when the protein analyte was in the stacking gel, and turned down to 90 V for about 3 h after it entered into the separating one.

After the removal of the mold (96-well plates) and a washing step, the gels were separately immersed in the five metal NPs (20 mL) on a shaker at room temperature for 3 h. Then the gel was illuminated by a bioimaging system for 30 min at an excitation of 312±10 nm, and the data was collected by adjusting 655±30 nm as the collected wavelength, with a 5 seconds exposure.

Table S2. Preparation of five colloidal metal NPs

Precursor (g)	Reducing Agent (mL)	Stabilizing Agent (g)	Reaction Temperature (℃)	Total Volume (mL)
$AuCl_3 \cdot HCl \cdot 4H_2O$ 0.0200	$N_2H_4 \cdot H_2O$ 0.05	PVP 0.0788	microwave heating	10
$AgNO_3$ 0.0085	$N_2H_4 \cdot H_2O$ 1	PVP 0.2250	50	20
$CuSO_4$ 0.0080	$N_2H_4 \cdot H_2O$ 1	PVP 0.2250	80	20
$NiSO_4 \cdot 6H_2O$ 0.0131	$N_2H_4 \cdot H_2O$ 1	PVP 0.2250	85	20
$CoSO_4 \cdot 7H_2O$ 0.0141	$N_2H_4 \cdot H_2O$ 1	PVP 0.2250	120	20

Table S3. Preparation of four colloidal metal NPs in a different way from the one in Table S2

Precursor (g)	Reducing Agent	Stabilizing Agent (g)	Reaction Temperature (℃)	Total Volume (mL)
$AuCl_3 \cdot HCl \cdot 4H_2O$ 0.0100	sodium citrate 0.05 g	——	100	100
$AgNO_3$ 0.0017	$NaBH_4$ 0.01g	PVP 0.1000	Room Temperature	100
$NiSO_4 \cdot 6H_2O$ 0.0131	$N_2H_4 \cdot H_2O$ 1 mL	——	85	20
$CoSO_4 \cdot 7H_2O$ 0.0141	$N_2H_4 \cdot H_2O$ 1 mL	——	120	20

3. Cu NPs-HSA Binding Experiments

The titrations of HSA with Cu NPs are carried out on a MicroCal VP-ITC calorimeter (Northampton, MA), and the data is analysed by a Windows-based Origin software package, which is also supplied by MicroCal. In the experiments, the reaction cell is totally filled (*i.e.*, no air spaces) with HSA solution (0.1 mM, 285 μL), and the titration injector syringe is also totally filled with the Cu NPs (0.5 mM, 300 μL). After some instrument operation, the titration injector syringe is inserted into the reaction cell. During the titration, the Cu NPs is titrated to HSA with 30 injections of 10 μL each spaced 2 min apart. During the spaced 2 min, the system can reach thermal equilibrium again. After the titration, the measured curve are formed and shown in the Figure 4A (the upper one), in which the x-coordinate represents the time of titration

and y-coordinate represents the heat of reaction. With the titration, the heat of reaction decreases gradually until the reaction tends to balance. At last, with the measured curve analysed by Origin software package, we can get a solid line in the bottom plot (Figure 4A) to get the thermodynamic parameters (ΔH, ΔS and K) and the titrant-to-sample molar ratio of near 0.25, which represents the best fit of our data to two-site equilibrium binding expression and is in accordance with the relevant report.[1]

Results and Discussion

1. The differentiation of the five metal NPs by the ten proteins

The differentiation of the five metal NPs can also be realised, according to the pattern variation of the sensor composed of the ten proteins. As can be seen in Figure S1A, each metal NPs has its own characteristic "fingerprint" map, which can be used to recognise the NPs. For instance, the Cu NPs are strongly emissive with the addition of HSA, catalase, lysozyme, EA and γ-globulins, whereas the Cu NPs has low FL with the other proteins; the Cu NPs is all rose red in the presence of papain, trypsin, hemoglobin human (Hb) and IgG, and is pale blue in the presence of the other proteins. By comparison, for Ag NPs, the FL is rather high with the addition of catalase and Hb while rather low with the other ones; and the colour is pale green with HSA, white with catalase, brick-red with Hb, bluish-white with lysozyme and EA, and violet with papain, trypsin, mucins, IgG and γ-globulins.

The quantitative analysis experiment is also carried out for the determination of the sensitivity of the sensor, through the analysis of the fluorescence intensity signal of proteins by the addition of nanoparticles at different concentrations, while keeping the concentration of the proteins constant. Here, lysozyme with Cu NPs as well as Hb with Ni NPs and Co NPs are taken as examples (Figure S2). The limit of detections of Cu NPs, Ni NPs and Co NPs are determined to be 0.0625 mM, 0.125 mM and 0.125 mM respectively. It can be seen in Figure S2a$_1$ that the calibration curve of Cu NPs bends towards to the concentrations axis at 0.625 mM, which means that the linear dynamic range of Cu NPs is from 0.0625 mM to 0.625 mM. Similarly, it can be determined that the linear dynamic range of Ni NPs is from 0.125 mM to 1.25 mM and that of Co NPs is from 0.125 mM to 2.5 mM.

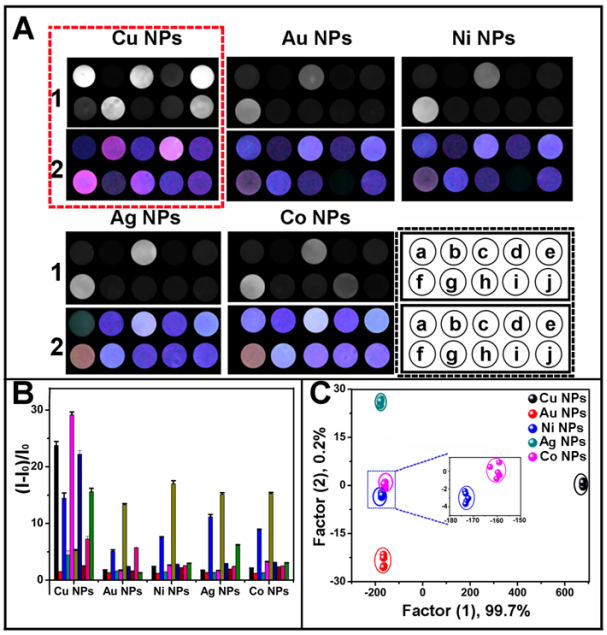

Figure S1. (A) FL intensity (1) and colour (2) difference maps for five metal NPs, with the addition of proteins (a: HSA, b: papain, c: catalase, d: trypsin, e: lysozyme, f: Hb, g: EA, h: mucins, i: IgG, j: γ-globulins). The concentration of each protein is 2 mg mL^{-1} and that of metal NPs is 2.5 mM, the excitation wavelength is 312±10 nm.(B) FL intensity change patterns of five metal NPs in the presence of ten proteins (the change patterns are acquired as an average of five parallel measurements). I_0 represents the average FL intensity values of the background, and I represents these of protein-NPs.(C) Canonical score plot of the first two factors of FL intensity response patterns, obtained through the sensor against five target metal NPs; the insert is partial enlargement of the plot.

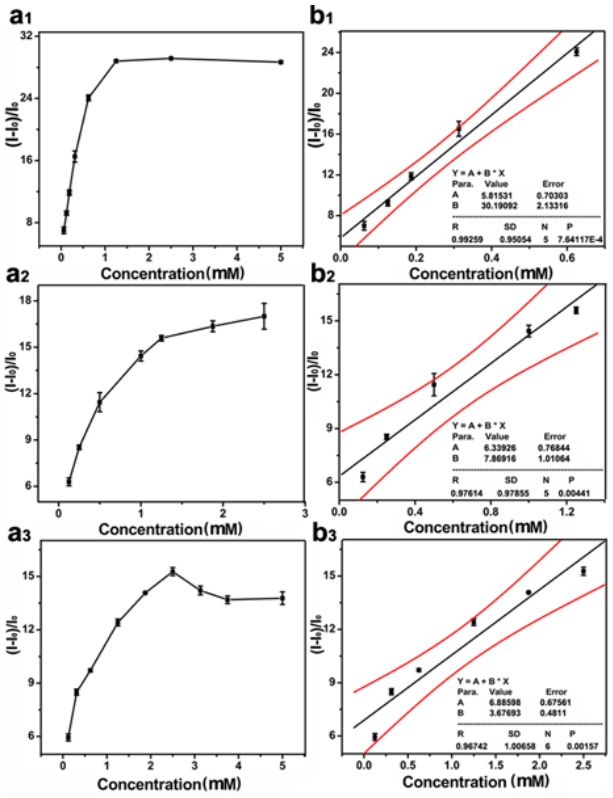

Figure S2 (a_1, a_2, a_3) FL intensity change with the variation of NPs concentrations (a_1: Cu NPs, a_2: Ni NPs, a_3: Co NPs) as an average of five parallel measurements. (b_1, b_2, b_3) Correlation of the concentrations of protein with the FL intensity in the linear dynamic range (b_1: Cu NPs, b_2: Ni NPs, b_3: Co NPs). The 95% confidence limit is marked in the figures. I_0 represents the average FL intensity values of the background, and I represents these of protein-NPs.

2. The discrimination of proteins by Linear Discriminant Analysis (LDA)

Table S4. The raw fluorescent data of five metal NPs with proteins in gel

Protein analytes	Control	Cu NPs		Au NPs		Ni NPs		Ag NPs		Co NPs	
	I_0	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$
HSA	42.6	1040	24.0	114.3	1.8	98.1	1.4	110.3	1.7	96.8	1.3
HSA	41.4	1053	24.3	116.3	1.8	101.6	1.4	114.6	1.8	98.4	1.4
HSA	43.7	1022	23.6	115.0	1.8	103.0	1.5	113.5	1.7	97.6	1.3
HSA	36.9	1003	23.1	110.2	1.7	101.7	1.4	111.3	1.7	99.0	1.4
HSA	40.8	991.8	22.9	113.9	1.7	99.4	1.4	107.9	1.6	93.3	1.2
Papain	41.1	100.3	1.4	92.4	1.2	101.3	1.5	93.4	1.3	101.9	1.5
Papain	41.3	104.3	1.5	91.4	1.2	104.2	1.5	96.3	1.3	111.4	1.7
Papain	40.2	100.2	1.4	95.0	1.3	85.8	1.3	98.6	1.4	94.1	1.3
Papain	42.4	107.5	1.6	89.6	1.2	92.0	1.2	94.1	1.3	113.8	1.8
Papain	43.4	107.1	1.6	98.6	1.4	99.3	1.4	90.7	1.2	118.0	1.8
Catalase	42.8	637.0	14.3	251.6	5.1	325.1	6.8	491.5	10.8	388.9	8.4
Catalase	41.5	645.3	14.5	263.7	5.3	327.6	6.9	500.0	11.0	380.8	8.2
Catalase	40.9	650.0	14.5	265.7	5.4	332.6	7.0	509.1	11.2	388.3	8.3
Catalase	42.3	693.1	14.7	243.5	4.9	321.3	6.7	494.8	10.9	390.3	8.4
Catalase	43.6	586.7	15.7	246.4	4.9	328.4	6.9	532.5	11.9	392.8	8.5
Trypsin	41.0	194.9	3.7	112.8	1.7	97.3	1.3	94.9	1.3	97.8	1.4
Trypsin	40.5	221.5	4.3	103.3	1.5	91.6	1.2	94.0	1.3	97.2	1.3
Trypsin	40.9	226.9	4.5	103.2	1.5	90.0	1.2	100.5	1.4	101.4	1.4
Trypsin	41.3	193.9	3.7	108.6	1.6	88.3	1.1	89.7	1.2	88.5	1.2
Trypsin	41.2	275.2	5.6	99.2	1.4	98.6	1.4	97.4	1.3	94.3	1.3
Lysozyme	40.5	1229.5	28.6	118.1	1.8	148.8	2.6	112.9	1.7	174.5	3.2
Lysozyme	43.0	1265.6	29.5	106.8	1.6	146.3	2.5	110.4	1.7	176.6	3.3
Lysozyme	42.6	1234.9	28.7	107.6	1.6	150.8	2.6	109.3	1.6	179.1	3.3
Lysozyme	42.7	1278.5	29.8	112.8	1.7	148.3	2.6	110.9	1.7	170.8	3.1
Lysozyme	42.0	1256.5	29.2	100.1	1.4	152.9	2.7	111.4	1.7	170.0	3.1
Hb	40.1	255.1	5.1	586.4	13.1	718.0	16.3	675.3	15.3	677.3	15.3
Hb	39.4	249.7	5.0	590.1	13.2	760.0	17.3	681.5	15.4	671.9	15.2
Hb	38.2	264.7	5.4	590.5	13.2	736.7	16.7	675.6	15.3	690.2	15.6
Hb	39.9	258.0	5.2	601.9	13.5	774.1	17.7	681.5	15.1	668.1	15.1
Hb	42.1	265.1	5.4	603.4	13.5	751.7	17.1	652.7	14.7	673.1	15.2
EA	42.7	949.9	21.9	144.4	2.5	159.1	2.8	166.4	3.0	173.7	3.2
EA	41.0	956.1	22.0	142.0	2.4	157.1	2.8	163.8	2.9	169.1	3.1
EA	40.8	961.5	22.1	142.2	2.4	150.0	2.6	161.2	2.9	173.3	3.2
EA	41.5	939.9	21.6	137.1	2.3	152.1	2.7	161.5	2.9	174.9	3.2
EA	40.6	1010.5	23.3	139.0	2.3	161.2	2.9	158.5	2.8	163.7	2.9

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Protein analytes	Control	Cu NPs		Au NPs		Ni NPs		Ag NPs		Co NPs	
	I_0	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$
Mucins	40.6	147.5	2.6	107.1	1.6	134.2	2.2	123.3	2.0	137.5	2.3
Mucins	41.3	146.3	2.5	106.1	1.6	130.0	2.1	124.0	2.0	140.0	2.4
Mucins	42.0	142.5	2.4	111.0	1.7	134.6	2.2	118.9	1.9	140.0	2.4
Mucins	43.1	149.2	2.6	109.6	1.6	130.5	2.1	123.0	2.0	140.8	2.4
Mucins	41.8	145.0	2.5	109.4	1.6	131.3	2.2	118.1	1.8	140.0	2.4
IgG	40.6	344.4	7.3	272.2	5.6	147.5	2.6	136.0	2.3	147.1	2.5
IgG	41.3	346.1	7.3	278.4	5.7	147.9	2.6	140.0	2.4	143.8	2.5
IgG	41.6	373.1	8.0	271.5	5.5	143.8	2.5	142.0	2.4	146.7	2.5
IgG	40.5	322.4	6.8	283.9	5.8	149.2	2.6	140.3	2.4	145.8	2.5
IgG	42.4	332.8	7.0	282.7	5.8	147.9	2.6	146.9	2.5	146.7	2.5
γ-globulins	41.8	657.3	14.8	91.7	1.2	161.2	2.9	292.9	6.0	167.9	3.0
γ-globulins	40.4	697.2	15.8	95.6	1.3	165.4	3.0	301.6	6.3	164.1	3.0
γ-globulins	42.3	670.2	15.1	95.8	1.3	161.6	2.9	293.7	6.1	169.1	3.1
γ-globulins	41.7	696.0	15.7	91.4	1.2	165.8	3.0	302.4	6.3	167.4	3.0
γ-globulins	40.8	723.8	16.4	94.0	1.3	160.8	2.9	292.0	6.0	162.5	2.9

3. The discrimination of HSA, BSA, denatured HSA and denatured BSA

To determine whether the sensor can be used to discriminate between proteins with similar properties, HSA, BSA, denatured HSA and denatured BSA are studied. The HSA and BSA are denatured by boiling in water for 1 h. It can be seen in Figure S3 that the patterns of the four proteins are nearly identical, indicating that the discrimination of the four proteins via this sensor is not possible.

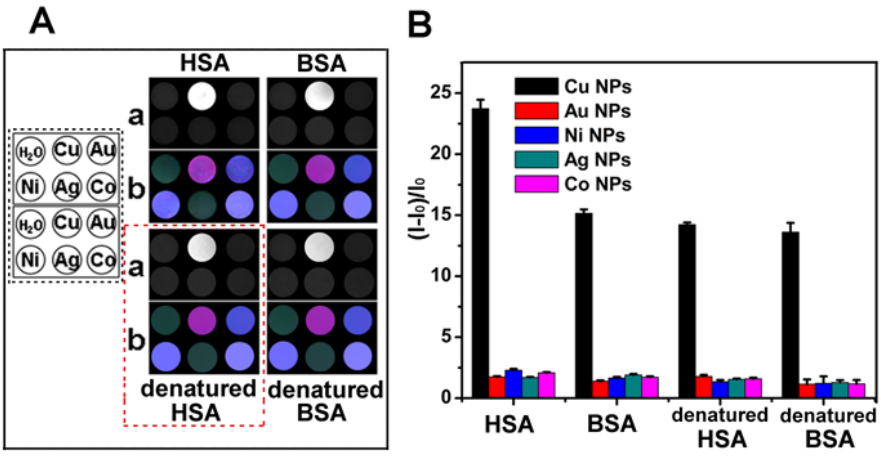

Figure S3. (A) FL intensity (a) and colour (b) difference maps for HSA, BSA, denatured HSA and denatured BSA, with and without the addition of metal NPs. The

concentration of each protein is 2 mg mL^{-1} and that of metal NPs is 2.5 mM, the excitation wavelength is 312±10 nm. (B) FL intensity change patterns of HSA, BSA, denatured HSA and denatured BSA in the presence of five metal NPs (the change patterns are acquired as an average of five parallel measurements). I_0 represents the average FL intensity values of the background, and I represents these of protein-NPs.

4. Quantitative analysis of the proteins using the sensor

The quantitative analysis experiment is also carried out for the determination of the sensitivity of the sensor, through the analysis of the fluorescence intensity signal of proteins at different concentrations by the addition of nanoparticles, while keeping the concentration of the nanoparticles constant. Here, HSA and lysozyme with Cu NPs as well as Hb with Ni NPs are taken as examples (Figure S4). The limit of detections of HSA, lysozyme and Hb are determined to be 0.288 μM, 1.39 μM and 0.310 μM respectively. It can be seen in Figure S4a_1 that the calibration curve of HSA bends towards to the concentrations axis at 28.8 μM, which means that the linear dynamic range of HSA is from 0.288 μM to 28.8 μM. Similarly, it can be determined that the linear dynamic range of lysozyme is from 1.39 μM to 69.4 μM and that of Hb is from 0.310 μM to 15.5 μM.

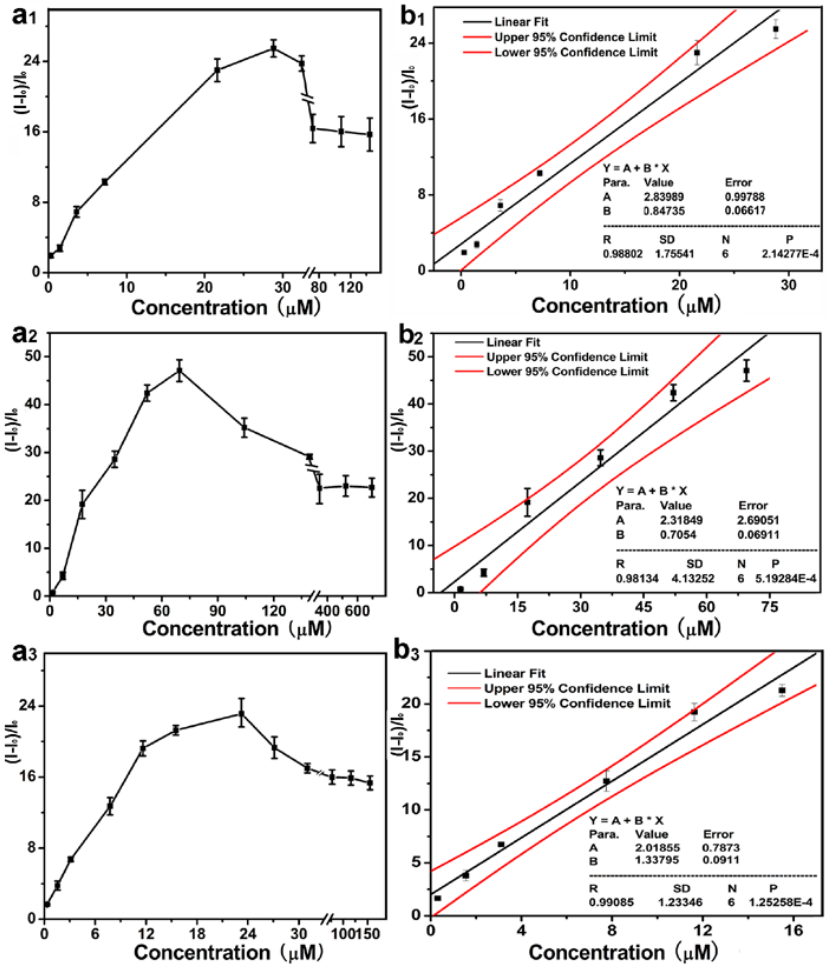

Figure S4. (a_1, a_2, a_3) FL intensity change with the variation in protein concentrations (a_1: HSA, a_2: lysozyme, a_3: Hb) as an average of five parallel measurements. (b_1, b_2, b_3) Correlation between the protein concentrations and the FL intensity in the linear dynamic range (b_1: HSA, b_2: lysozyme, b_3: Hb). The 95% confidence limit is marked in the figures. I_0 represents the average FL intensity values of the background, and I represents these of protein-NPs.

5. The discrimination of proteins by metal NPs after changing the NPs synthetic method

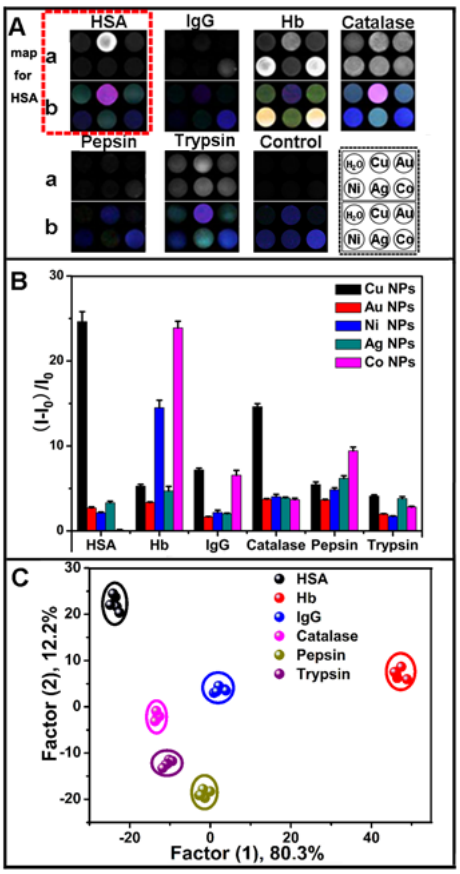

Figure S5. (A) FL intensity (a) and colour (b) difference maps for six proteins, with and without the addition of metal NPs. The concentration of each protein is 2 mg mL^{-1}, and the excitation wavelength is 312±10 nm. (B) FL intensity change patterns of ten proteins in the presence of five metal NPs (the change patterns are acquired as an average of five parallel measurements). I_0 represents the average FL intensity values of the background, and I represents these of protein-NPs.(C) Canonical score plot of the first two factors of FL intensity response patterns, obtained through the sensor against six target proteins.

Table 5. The raw fluorescent data of five metal NPs with six proteins in gel

Protein analytes	Control	Cu NPs		Au NPs		Ni NPs		Ag NPs		Co NPs	
	I_0	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$	I	$(I-I_0)/I_0$
HSA	39.4	1035	23.9	150.4	2.6	128.8	2.1	178.9	3.3	46.3	0.11
HSA	41.4	1089	25.2	159.6	2.8	125.2	2.0	187.8	3.5	44.8	0.08
HSA	40.1	1102	25.5	159.7	2.8	135.6	2.3	170.5	3.1	46.8	0.13
HSA	36.9	994	22.9	148.7	2.6	132.4	2.2	170.9	3.1	40.7	-0.02
HSA	43.7	1105	25.6	152.5	2.7	131.3	2.2	185.9	3.5	48.6	0.17
Hb	38.2	255.7	5.2	181.2	3.4	632.0	14.2	241.6	4.8	1037	24.0
Hb	40.6	260.4	5.3	179.5	3.3	624.0	14.0	221.7	4.3	1014	23.4
Hb	42.1	270.8	5.5	176.8	3.3	684.6	15.5	243.0	4.8	1027	23.7
Hb	42.6	249.4	5.0	185.6	3.5	680.3	15.4	268.3	4.3	1088	25.2
Hb	40.1	268.3	5.5	178.3	3.3	600.6	13.5	210.2	4.8	1006	23.2
IgG	42.3	340.8	7.2	112.2	1.7	121.3	1.9	122.3	1.9	340.6	7.2
IgG	41.3	332	7.0	112.1	1.7	140.0	2.4	127.1	2.1	339.1	7.2
IgG	41.6	351.8	7.5	108.4	1.6	141.2	2.4	127.0	2.1	305.2	6.3
IgG	42.4	344.9	7.3	112.2	1.7	139.7	2.4	129.4	2.1	289.9	6.0
IgG	42.8	329.7	6.9	106.8	1.6	119.9	1.9	123.2	2.0	297.8	6.2
Catalase	40.8	653.4	14.7	201.2	3.8	213.1	4.1	200.0	3.8	192.5	3.6
Catalase	42.6	642.8	14.5	194.6	3.7	219.5	4.3	203.9	3.9	191.0	3.6
Catalase	40.9	633.6	14.2	192.9	3.6	219.9	4.3	204.6	3.9	197.4	3.8
Catalase	42.3	673.1	15.2	194.4	3.7	200.4	3.8	208.9	4.0	205.4	3.9
Catalase	43.6	642.1	14.5	198.8	3.8	194.3	3.7	194.8	3.7	186.6	3.5
Pepsin	40.9	251.7	5.1	194.1	3.7	241.9	4.8	296.6	6.1	436.3	9.5
Pepsin	41.5	275.4	5.6	196.6	3.7	244.0	4.9	291.3	6.0	458.6	10.0
Pepsin	40.5	273.5	5.6	194.3	3.7	257.0	5.2	302.6	6.3	412.6	8.9
Pepsin	39.9	282.1	5.8	192.5	3.6	242.7	4.8	286.6	5.9	419.4	9.1
Pepsin	41.2	257.7	5.2	186.3	3.5	230.0	4.5	318.2	6.7	440.5	9.6
Trypsin	41.0	215.4	4.2	121.9	1.9	116.4	1.8	197.8	3.8	160.5	2.9
Trypsin	40.5	206.3	4.0	123.9	2.0	114.0	1.7	185.4	3.5	162.4	2.9
Trypsin	41.3	217.9	4.2	126.2	2.0	111.3	1.7	197.4	3.7	160.0	2.8
Trypsin	40.6	204.5	3.9	121.4	1.9	109.1	1.6	207.5	4.0	158.0	2.8
Trypsin	42.4	216.0	4.2	127.0	2.1	112.4	1.7	211.0	4.1	152.2	2.7

6. The mechanism of the variation in the FL properties of the metal NPs

6.1. The fluorescence resonance energy transfer (FRET)

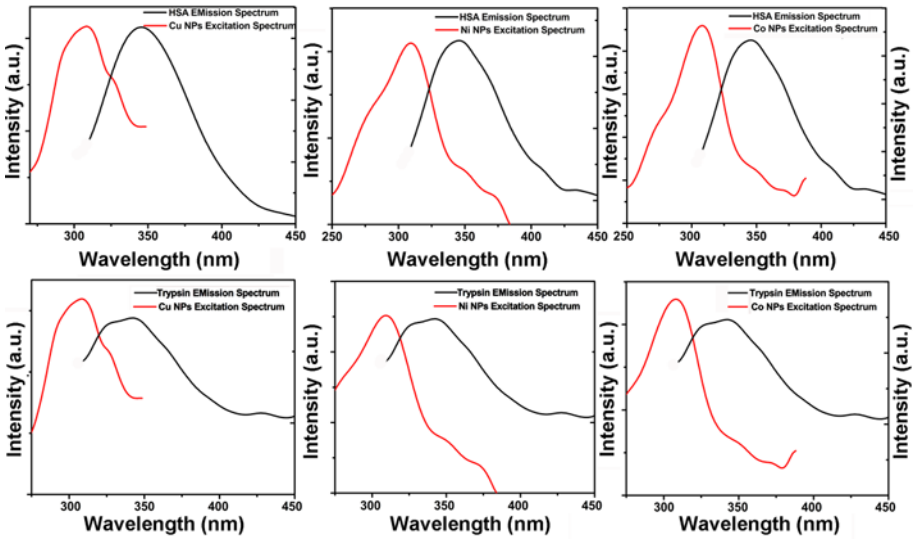

Figure S6. Emission spectra of free proteins (excited at 312 nm) and excitation spectra of the metal NPs.

Here, the emission spectra of free proteins (excited at 312 nm) and the FL excitation spectra of the metal NPs in solution are detected. As can be seen in Figure S6 that there is much overlap of the emission band of proteins with the excitation band of NPs, which suggests that at an excitation of 312 nm, the energy of proteins can be absorbed by the NPs and consequently the NPs can be excited to be luminescent. This may suggest the possibility of fluorescence resonance energy transfer (FRET) from the proteins to the NPs.

6.2. The exclusion of gel components function

In this sensor, it is assumed that the gel is just used to immobilise the proteins and metal NPs based on its 3-D polymer networks,[2] and has no effect on the metal NPs. To validate this hypothesis, the five metal NPs are simply mixed with the gel components (TEMED, $(NH_4)_2S_2O_8$, gel stock solution) individually, and the ratio of metal NPs to the gel components is the same as in the gel. As shown in Figure S7A, the FL properties of five metal NPs with gel components are almost identical, and the intensity of the metal NPs can not be enhanced. Photographs are taken under ultraviolet light, as shown in Figure S7B, and there are no colour changes, indicating a

red-shifting effect on metal NPs. Therefore, there are not effective interactions between the gel components and the metal NPs to affect the FL properties.

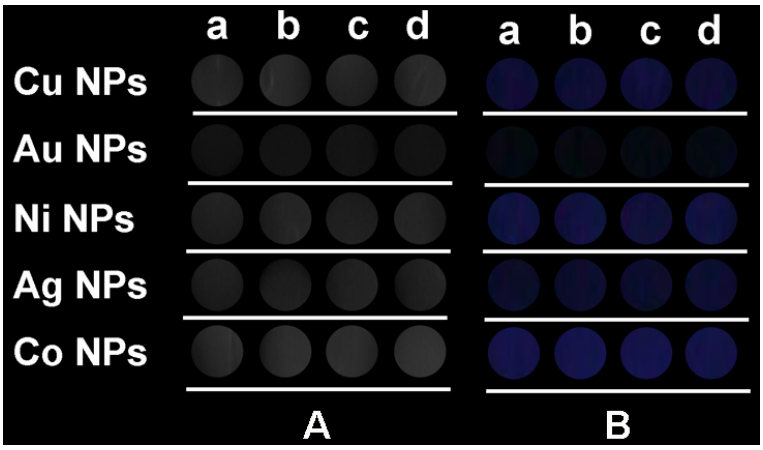

Figure S7. FL change profiles of five metal NPs (Cu, Au, Ni, Ag and Co NPs) with or without the addition of the gel components (a: distilled water, b: TEMED, c: $(NH_4)_2S_2O_8$, d: gel stock solution), with exciation at 312±10 nm.

(A) FL intensity images

(B) FL colour images

7. The discrimination of serum samples by the metal NPs based on gel

The FL colours of NPs are transformed into RGB (red, green and blue) numerical values and the values for the red colour are selected. The data for the red colour are then subjected to LDA to better discriminate the serum samples (Figure S8).

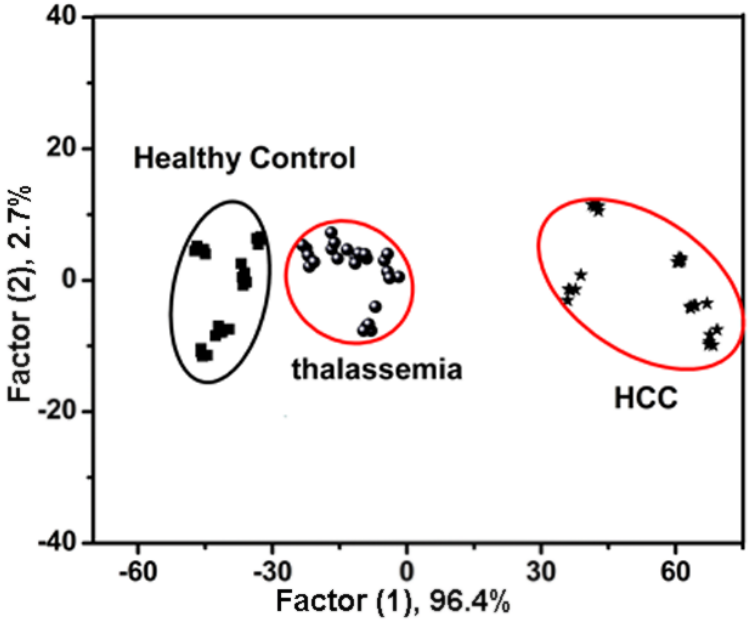

Figure S8. Canonical score plot of the first two factors of FL colour response patterns, obtained through the sensor against five normal sera, five hepatocellular carcinoma (HCC) sera and five thalassemia sera.

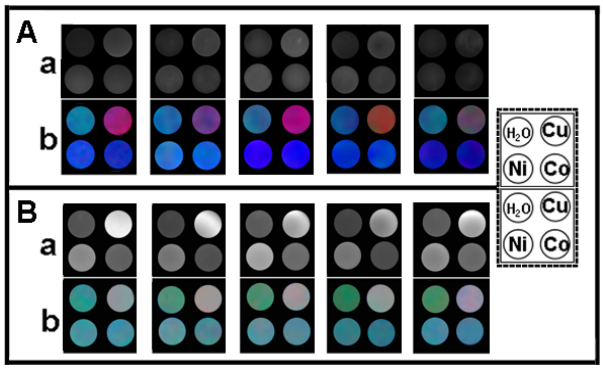

Figure S9. FL intensity (a) and colour (b) difference maps for sera from healthy men (A) and thalassemia patients (B), with and without the addition of metal NPs.

Refererence

[1] Y. Zhang, and D. E. Wilcox, *J. BiolInorg. Chem.*, 2002, **7**, 327.

[2] D. Kim, K. Karns, S. Q. Tia, M. He and A. E. Herr, *Anal. Chem.*, 2012, **84**, 2533.