Supplementary Information

Nanoplasmonic probe as a triple channel colorimetric sensor array for proteins discrimination

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Experimental section

Materials and instruments The oligonucleotide was purchased from Sangon Biotech Co., Ltd. (Shanghai, China): 5’-AAA AAA AAA AAA AAA AAA AAA-3’ (named A21). Sodium chloride and Chloroauric acid ($HAuCl_4$) were purchased from Sinopharm Chemical Reagent Company (Beijing, China). Sodium citrate was purchased from Xilong Chemical Co., Ltd. (Shantou, China). Hydroxylamine hydrochloride ($NH_2OH·HCl$) solution 50% w/v in water was purchased from Alfa Aesar Tianjing Chemical Reagent Company (Tianjing, China). All of the proteins were purchased from Beijing Biodee Biotechnology Co., Ltd. (Beijing, China). All the other reagents were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All the reagents were of at least analytical grade and were used without any other further purification. The oligonucletotide solution in this work was obtained by diluting the stock solution with a 20 mM Tris-HCl buffer (pH 7.4) which contained 140 mM NaCl and 5mM KCl. The protein solutions were prepared with deionized water.

UV-visible absorption spectra were recorded on a Scinco S3100 UV-Vis Spectrophotometer (Scientific Instrument Company, Daejeon City, Korea). The resonance light scattering (RLS) signal was conducted on an Edinburgh FLS-920 spectrophotometer. Water used throughout the experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Synthesis of AuNPs Water-soluble AuNPs with the diameter of 13 nm were synthesized according to a literature method.[1] The 200mL of 1 mM $HAuCl_4$ solution was put in a three-necked flask and was heated to reflux for 5 minutes under vigorous stirring, 20 mL of 38.8 mM sodium citrate solution was then immediately added into the boiling solution. With a color change from light yellow to wine red. After the mixture was allowed to reflux for 15 minutes, the heating was stopped, but stirring was continued for an additional 15 minutes, then the solution was cooled to the room temperature. After that, the solution was filtered through a 0.22 μm membrane filter and stored in a fridge of 4℃ before being used. The concentration of AuNPs (13 nm in diameter) was measured by UV-Vis spectroscopy based on an extinction coefficient of 2.7×10^{8} $M^{-1}\cdot cm^{-1}$ at 520 nm.

The fabrication of the multidimensional sensing system A single-strand oligonucleotide (A21) was designed to construct the sensing system. The oligonucleotide (11 μL of 1.0 μM) was added into the as-prepared AuNPs solution (137 μL of 10.0 nM). The mixture was incubated for 0.5 hour to form A21-AuNPs conjugates as sensing element solution on a shaker at room temperature, which was equally divided into three fractions for the multidimensional sensing proteins.

Discrimination of proteins The as-prepared conjugates of A21-AuNPs were employed to this multidimensional sensor array for the detection of proteins. The protein solution (11 μL of 1.0 μM) (or blank solution) was mixed with as-prepared A21-AuNPs conjugates solution and incubated for about 30 minutes at room temperature. Then, this sensing platform is involved three processes. In the first process, the mixture was diluted to a total of 220 μL. The resonance light scattering (RLS) signal at 650 nm was recorded. The final concentrations of AuNPs, DNA and protein, were 6.25 nM, 50 nM and 50 nM, respectively. In the later process, the reductant NH_2OH (1.0 M, 11 μL) and oxidant $HAuCl_4$ (1.0 mM, 11μL) was added into the same mixture to induce the growth of AuNPs, continuing to incubate for half an hour. Subsequently, the mixture solution was also diluted with deionized water to 220 μL. Then the absorbance of the mixture at 520 nm and 620 nm were recorded. The final concentrations of NH_2OH and $HAuCl_4$, were 50 mM, and 50 μM, respectively. The last sensing process was the same as the second detection except that the NH_2OH and $HAuCl_4$ were replaced with NaCl at the final concentration of 25 mM. In the multidimensional sensing system, the changes in the three optical signals of AuNPs lead to the discrimination of proteins.

Real sample detection In tests with human urine samples, morning urine from a health volunteer was collected and centrifuged for 10 minutes (14000 rpm) to remove the insoluble matrix. Urine was diluted with deionized water to 50% (v/v). All other conditions were equivalent to the above tests except there is no additional salt added into the urine samples.

Data analysis All measurements were repeated to generate six replicates for each protein. To eliminate the potential bias caused by the difference in the original signal intensity of sensing system, the raw data were first normalized (k/k_0). The data was analyzed by linear discriminant analysis (LDA) applying Mahalanobis clustering analysis and hierarchical cluster analysis (HCA), using the software SYSTAT V13.0 and SPSS V16.0, respectively. LDA is a type of statistic software to recognize the linear combination of features that differentiate two or more classes of object or event, which can transforms the raw response patterns to canonical patterns, and maximizes the ratio of between-class variance to within-class variance, thereby it can enable maximal separability according to the preassigned grouping.[2] HCA is a model-free method based on the grouping of the analyte vectors according to their spatial distances in their full vector space. [3] After the analysis, three canonical factors were generated that represented linear combinations of the response matrices, obtained from the absorbance response patterns (three channels × thirteen proteins × six replicates). The first two or three most significant discrimination factors were used to generate a two or three-dimensional plot.

Table S1. Basic properties and manufacturers of proteins

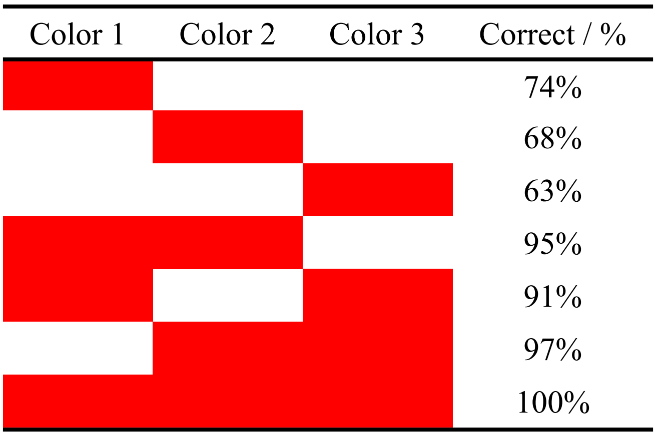
Protein	MW/kDa	pI	Metal	Company	Purity
Bovine serum albumin (BSA)	66.3	4.8	No	Amresco	>98%
Concanavalin A (Con-A)	102	~7.1	NO	BioDee	>90%
Cytochrome C (Cyt-C)	12	10.8	Yes	Amresco	>98%
Egg white albumin (EA)	44.3	4.6	No	Sigma	>95%
Hemoglobin (Hem) from bovine blood	64.5	6.8	Yes	Sigma	>95%
Horseradish Peroxidase (HRP)	~40	~5	Yes	BioDee	>95%
Human serum albumin (HSA)	69.4	5.2	No	Sigma	>95%
Immunoglobulin G (IgG) from pig blood	150	7.5	No	Sigma	>90%
Lysozyme (Lys)	14.4	11.0	No	Amresco	>95%
myoglobin (Myo)	17	7.2	Yes	Sigma	>95%
Pepsin (Pep) 1:3000	35	1~2.5	No	Sigma	>85%
Transferrin (TRF) from human blood	~75	5.6	Yes	Sigma	>98%
Trypsin (Try) 1:250	24	10.5	No	Amresco	>95%

Table S2. Training matrix of the response patterns against various proteins at 50 nM

Proteins	Color 1	Color 2	Color 3
BSA	0.619	0.523	0.266
BSA	0.618	0.522	0.264
BSA	0.618	0.520	0.247
BSA	0.610	0.503	0.247
BSA	0.604	0.501	0.246
BSA	0.593	0.499	0.238
Con-A	1.088	0.698	2.145
Con-A	1.084	0.692	2.141
Con-A	1.082	0.691	2.141
Con-A	1.080	0.690	2.141
Con-A	1.075	0.687	2.138
Con-A	1.060	0.686	2.137
Cyt-C	1.110	0.998	2.143
Cyt-C	1.101	0.996	2.142
Cyt-C	1.088	0.991	2.142
Cyt-C	1.079	0.991	2.141
Cyt-C	1.076	0.990	2.133
Cyt-C	1.071	0.988	2.132
EA	1.046	0.898	0.228
EA	1.044	0.884	0.227
EA	1.031	0.879	0.226
EA	1.027	0.872	0.225
EA	1.021	0.856	0.224
EA	1.014	0.838	0.223
Hem	1.291	1.076	1.995
Hem	1.290	1.075	1.991
Hem	1.286	1.051	1.990
Hem	1.278	1.046	1.913
Hem	1.269	1.013	1.911
Hem	1.235	1.005	1.895
HRP	1.242	1.136	0.435
HRP	1.236	1.128	0.432

HRP	1.233	1.113	0.429
HRP	1.222	1.109	0.426
HRP	1.221	1.109	0.420
HRP	1.197	1.098	0.414
HSA	0.668	0.566	0.248
HSA	0.662	0.553	0.248
HSA	0.662	0.545	0.246
HSA	0.660	0.545	0.245
HSA	0.660	0.541	0.243
HSA	0.655	0.537	0.242
IgG	1.217	0.902	1.983
IgG	1.213	0.882	1.964
IgG	1.206	0.870	1.947
IgG	1.205	0.863	1.945
IgG	1.201	0.862	1.917
IgG	1.192	0.849	1.911
Lys	0.923	1.011	1.899
Lys	0.906	1.000	1.898
Lys	0.892	0.991	1.875
Lys	0.889	0.974	1.873
Lys	0.878	0.970	1.866
Lys	0.872	0.965	1.864
Myo	1.061	0.974	1.591
Myo	1.047	0.973	1.587
Myo	1.045	0.968	1.571
Myo	1.045	0.967	1.548
Myo	1.031	0.960	1.546
Myo	1.028	0.951	1.542
Pep	0.729	0.817	0.262
Pep	0.705	0.816	0.246
Pep	0.704	0.796	0.243
Pep	0.693	0.792	0.242
Pep	0.659	0.790	0.242
Pep	0.658	0.787	0.241
TRF	0.902	0.976	0.197

TRF	0.884	0.970	0.197
TRF	0.861	0.960	0.196
TRF	0.856	0.958	0.196
TRF	0.848	0.956	0.194
TRF	0.834	0.942	0.193
Try	0.983	1.011	0.191
Try	0.976	0.998	0.190
Try	0.975	0.997	0.190
Try	0.973	0.997	0.189
Try	0.957	0.995	0.187
Try	0.951	0.992	0.186



Color 1	Color 2	Color 3	Correct / %
🟥			74%
	🟥		68%
		🟥	63%
🟥	🟥		95%
🟥		🟥	91%
	🟥	🟥	97%
🟥	🟥	🟥	100%

Figure S1. Jackknifed classification matrix obtained using LDA for sensing channels for the 13 proteins.

Table S3. LDA classification accuracy of proteins (50 nM) using the triple channel colorimetric sensor array. The values are taken from the Jackknifed classification matrix based on raw data listed in **Table S2**

Proteins	Color 1	Color 2	Color 3	Color 1 and 2	Color 1 and 3	Color 2 and 3	Triple Channels
BSA	100	100	33	100	100	100	100
Con-A	50	100	67	100	67	100	100
Cyt-C	50	50	33	100	50	100	100
EA	67	0	100	100	100	100	100
HRP	83	100	100	100	100	100	100
HSA	100	100	33	100	100	100	100
Hem	83	67	0	100	100	67	100
IgG	83	0	17	100	100	100	100
Lys	67	0	100	67	100	100	100
Myo	50	67	100	100	100	100	100
Pep	67	100	17	100	67	100	100
TRF	67	67	100	67	100	100	100
Try	100	67	100	100	100	100	100
Total	74	63	62	95	91	97	100

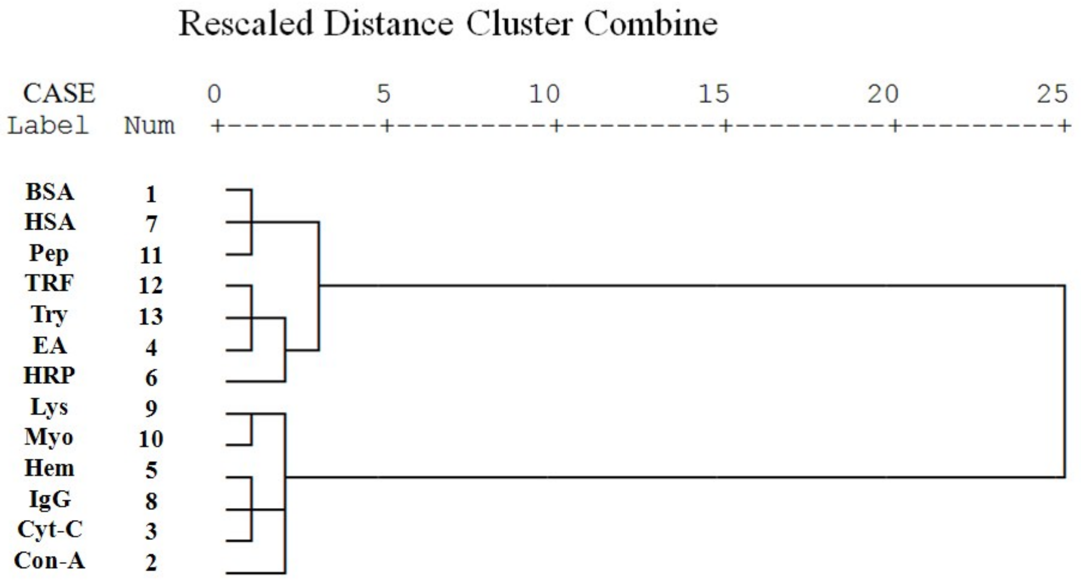


Figure S2. Hierarchical cluster analysis dendrogram for 13 protein analytes at 50 nM， all experiments were run in six replicates.

Table S4. Identification of unknown protein samples at 50 nM using the triple channel colorimetric sensor array

	Color 1	Color 2	Color 3	Identification	Verification
1	0.660	0.527	0.288	BSA	BSA
2	0.642	0.549	0.250	HSA	HSA
3	0.983	1.027	1.904	Lys	Lys
4	1.012	0.949	1.529	Myo	Myo
5	0.813	0.985	0.205	TRF	TRF
6	1.066	1.013	2.125	Cyt-C	Cyt-C
7	1.060	0.723	2.127	Con-A	Con-A
8	1.225	0.934	1.941	IgG	IgG
9	1.276	1.158	0.414	HRP	HRP
10	0.931	0.852	0.222	EA	EA
11	0.757	0.844	0.234	Pep	Pep
12	0.628	0.559	0.242	HSA	HSA
13	1.176	0.945	1.769	Hem	Hem
14	0.967	1.017	0.197	Try	Try
15	1.054	0.653	2.102	Con-A	Con-A
16	1.182	0.919	1.881	IgG	IgG
17	0.794	0.886	0.192	TRF	TRF
18	1.097	0.985	1.671	Myo	Myo
19	0.832	0.927	1.849	Lys	Lys
20	0.798	0.857	0.263	Pep	Pep
21	0.589	0.478	0.235	BSA	BSA
22	0.952	1.013	0.193	Try	Try
23	1.191	0.945	1.812	Hem	Hem
24	1.111	1.022	2.127	Cyt-C	Cyt-C
25	1.115	0.756	2.148	Con-A	Con-A
26	1.062	1.003	2.124	Cyt-C	Cyt-C
27	1.062	0.928	0.236	EA	EA
28	0.671	0.543	0.254	HSA	HSA
29	0.980	0.950	0.185	Try	Try
30	0.701	0.771	0.234	Pep	Pep
31	1.080	0.745	2.131	Con-A	Con-A

32	1.133	0.905	1.859	IgG	IgG
33	0.812	0.927	0.200	TRF	TRF
34	1.188	1.070	0.398	HRP	HRP
35	0.974	0.922	0.235	EA	EA
36	0.650	0.525	0.236	HSA	HSA
37	0.969	0.962	0.186	Try	Try
38	1.171	0.930	1.768	Hem	Hem
39	1.125	0.987	1.673	Myo	Myo
40	1.181	0.915	1.876	IgG	IgG
41	1.112	1.023	2.144	Cyt-C	Cyt-C
42	0.802	0.926	0.193	TRF	TRF
43	1.004	1.033	1.924	Lys	Lys
44	1.192	1.151	0.402	HRP	HRP
45	0.568	0.471	0.231	BSA	BSA
46	0.641	0.711	0.232	Pep	Pep
47	0.961	0.961	1.854	Lys	Lys
48	0.906	0.815	0.219	EA	EA
49	0.923	0.943	1.508	Myo	Myo
50	1.182	1.062	0.397	HRP	HRP
51	1.301	0.987	1.855	Hem	Hem
52	0.631	0.548	0.249	BSA	BSA

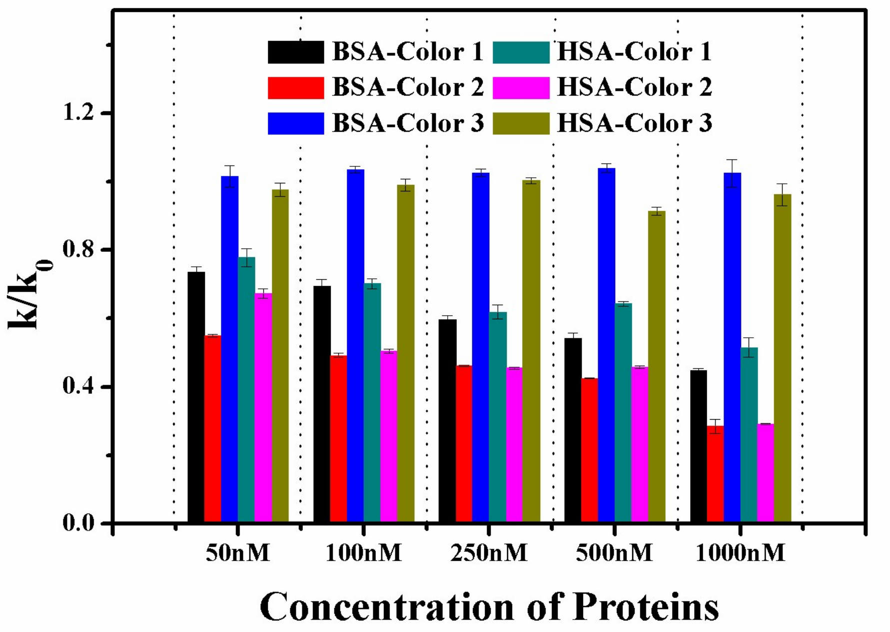


Figure S3. Fingerprints of HSA and BSA at various concentrations based on the patterns of the corresponding values of k/k_0 obtained from the triple channel colorimetric sensor array.

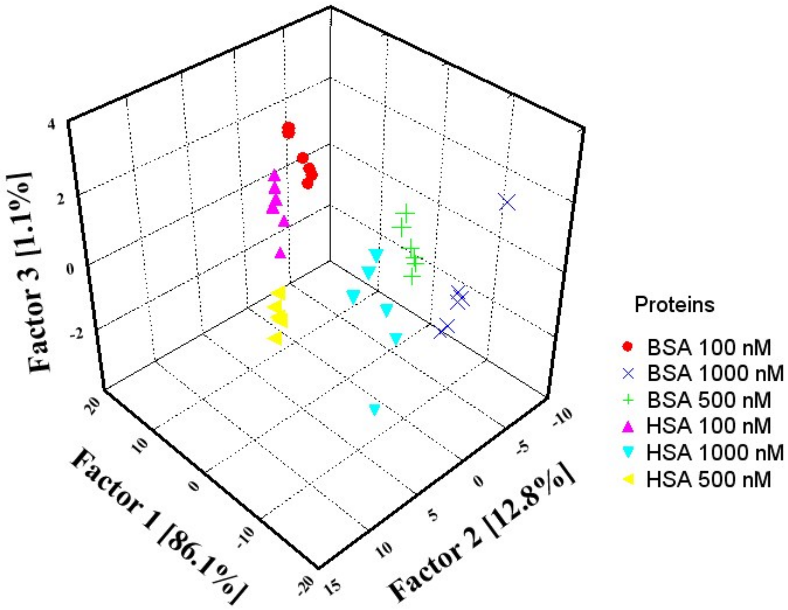


Figure S4. Canonical score plot for the three-channel patterns as obtained from LDA for BSA and HSA at different concentrations (100, 500 and 1000nM).

Table S5. Training matrix of the response patterns against BSA and HSA at various concentrations

Proteins	**Color 1**	**Color 2**	**Color 3**
BSA 100 nM	0.677	0.485	1.022
BSA 100 nM	0.677	0.486	1.030
BSA 100 nM	0.683	0.490	1.033
BSA 100 nM	0.694	0.495	1.036
BSA 100 nM	0.716	0.498	1.043
BSA 100 nM	0.718	0.499	1.046
HSA 100 nM	0.680	0.501	0.961
HSA 100 nM	0.689	0.501	0.985
HSA 100 nM	0.704	0.501	0.987
HSA 100 nM	0.705	0.503	0.994
HSA 100 nM	0.714	0.513	1.002
HSA 100 nM	0.719	0.514	1.010
BSA 500 nM	0.525	0.423	1.022
BSA 500 nM	0.528	0.425	1.033
BSA 500 nM	0.532	0.425	1.036
BSA 500 nM	0.541	0.426	1.040
BSA 500 nM	0.559	0.426	1.047
BSA 500 nM	0.562	0.429	1.059
HSA 500 nM	0.636	0.453	0.896
HSA 500 nM	0.638	0.453	0.912
HSA 500 nM	0.640	0.459	0.912
HSA 500 nM	0.644	0.460	0.913
HSA 500 nM	0.651	0.460	0.918
HSA 500 nM	0.654	0.462	0.930
BSA 1000 nM	0.444	0.269	1.019
BSA 1000 nM	0.447	0.274	1.100
BSA 1000 nM	0.453	0.305	0.991
BSA 1000 nM	0.456	0.313	1.027
BSA 1000 nM	0.448	0.282	1.016
BSA 1000 nM	0.444	0.266	0.994
HSA 1000 nM	0.476	0.290	0.902
HSA 1000 nM	0.538	0.292	0.983
HSA 1000 nM	0.542	0.295	0.961
HSA 1000 nM	0.539	0.294	0.997
HSA 1000 nM	0.508	0.291	0.971
HSA 1000 nM	0.488	0.290	0.959

Table S6. Identification of unknown protein samples at various concentrations using the triple channel colorimetric sensor array

	Color 1	Color 2	Color 3	Identification	Verification
1	0.709	0.501	1.004	HAS 100 nM	HAS 100 nM
2	0.497	0.274	1.220	BSA 1000 nM	BSA 1000 nM
3	0.608	0.480	0.907	HSA 500 nM	HSA 500 nM
4	0.508	0.291	0.942	HSA 1000 nM	HSA 1000 nM
5	0.669	0.490	1.051	BSA 100 nM	BSA 100 nM
6	0.621	0.448	0.995	HSA 500 nM	HSA 500 nM
7	0.542	0.295	0.923	HSA 1000 nM	HSA 1000 nM
8	0.560	0.428	1.082	BSA 500 nM	BSA 500 nM
9	0.636	0.483	1.061	BSA 100 nM	BSA 100 nM
10	0.577	0.428	1.066	BSA 500 nM	BSA 500 nM
11	0.506	0.313	1.074	BSA 1000 nM	BSA 1000 nM
12	0.494	0.269	1.058	BSA 1000 nM	BSA 1000 nM
13	0.538	0.292	0.966	HSA 1000 nM	HSA 1000 nM
14	0.760	0.506	0.988	HAS 100 nM	HAS 100 nM
15	0.728	0.502	1.021	HSA 100 nM	HSA 100 nM
16	0.682	0.494	1.034	BSA 100 nM	BSA 100 nM
17	0.571	0.447	0.912	HSA 500 nM	HSA 500 nM
18	0.558	0.427	1.090	BSA 500 nM	BSA 500 nM

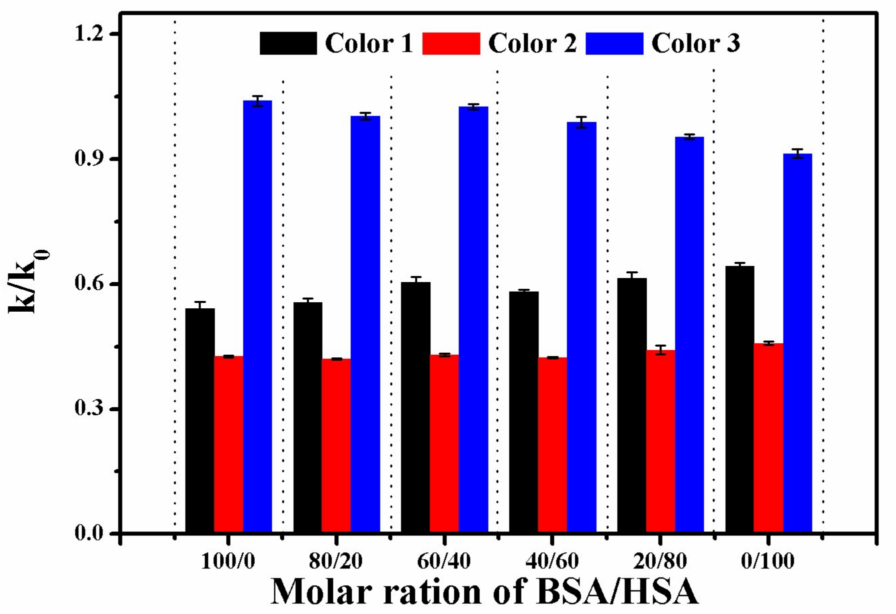


Figure S5. Fingerprints of BSA and HSA at different molar ratios (total protein concentration: 500 nM) based on the patterns of the corresponding values of k/k_0 obtained from the triple channel colorimetric sensor array.

Table S7. Training matrix of the response patterns against BSA and HSA at different molar ratios (total protein concentration: 500 nM)

Samples	Color 1	Color 2	Color 3
100% BSA	0.525	0.423	1.022
100% BSA	0.528	0.425	1.033
100% BSA	0.532	0.425	1.036
100% BSA	0.541	0.426	1.040
100% BSA	0.559	0.426	1.047
100% BSA	0.562	0.429	1.059
80% BSA + 20% HSA	0.543	0.418	0.993
80% BSA + 20% HSA	0.549	0.419	0.995
80% BSA + 20% HSA	0.558	0.420	0.999
80% BSA + 20% HSA	0.563	0.420	1.010
80% BSA + 20% HSA	0.563	0.421	1.011
80% BSA + 20% HSA	0.564	0.421	1.013
60% BSA + 40% HSA	0.588	0.426	1.019
60% BSA + 40% HSA	0.598	0.428	1.020
60% BSA + 40% HSA	0.606	0.429	1.022
60% BSA + 40% HSA	0.607	0.430	1.028
60% BSA + 40% HSA	0.609	0.430	1.032
60% BSA + 40% HSA	0.622	0.435	1.033
40% BSA + 60% HSA	0.576	0.422	0.964
40% BSA + 60% HSA	0.578	0.422	0.987
40% BSA + 60% HSA	0.583	0.422	0.989
40% BSA + 60% HSA	0.586	0.423	0.995
40% BSA + 60% HSA	0.586	0.425	0.998
40% BSA + 60% HSA	0.587	0.427	1.001
20% BSA + 80% HSA	0.594	0.430	0.946
20% BSA + 80% HSA	0.600	0.435	0.947
20% BSA + 80% HSA	0.609	0.435	0.955
20% BSA + 80% HSA	0.621	0.448	0.955
20% BSA + 80% HSA	0.624	0.452	0.959
20% BSA + 80% HSA	0.634	0.452	0.960
100% HSA	0.636	0.453	0.896

100% HSA	0.638	0.453	0.912
100% HSA	0.640	0.459	0.912
100% HSA	0.644	0.460	0.913
100% HSA	0.651	0.460	0.918
100% HSA	0.654	0.462	0.930

Table S8. Identification of unknown protein samples at different molar ratios using the triple channel colorimetric sensor array

Samples	Color 1	Color 2	Color 3	Identification	Verification
1	0.558	0.466	1.045	100% BSA	100% BSA
2	0.615	0.483	0.918	100% HSA	100% HSA
3	0.598	0.434	0.998	40% BSA + 60% HSA	40% BSA + 60% HSA
4	0.583	0.433	1.020	80% BSA + 20% HSA	80% BSA + 20% HSA
5	0.597	0.415	1.014	60% BSA + 40% HSA	60% BSA + 40% HSA
6	0.621	0.485	0.913	100% HSA	100% HSA
7	0.571	0.429	0.995	80% BSA + 20% HSA	80% BSA + 20% HSA
8	0.570	0.482	1.052	100% BSA	100% BSA
9	0.618	0.468	0.955	20% BSA + 80% HSA	20% BSA + 80% HSA
10	0.587	0.423	0.987	40% BSA + 60% HSA	40% BSA + 60% HSA
11	0.594	0.434	1.022	60% BSA + 40% HSA	60% BSA + 40% HSA
12	0.577	0.484	1.064	100% BSA	100% BSA
13	0.671	0.471	0.930	100% HSA	100% HSA
14	0.609	0.424	1.001	40% BSA + 60% HSA	40% BSA + 60% HSA
15	0.582	0.412	1.005	60% BSA + 40% HSA	60% BSA + 40% HSA
16	0.577	0.449	0.989	80% BSA + 20% HSA	80% BSA + 20% HSA
17	0.591	0.503	0.959	20% BSA + 80% HSA	20% BSA + 80% HSA
18	0.608	0.480	0.912	100% HSA	100% HSA
19	0.568	0.427	0.989	40% BSA + 60% HSA	40% BSA + 60% HSA
20	0.579	0.504	0.960	20% BSA + 80% HSA	20% BSA + 80% HSA
21	0.604	0.469	0.955	20% BSA + 80% HSA	20% BSA + 80% HSA
22	0.560	0.480	1.041	100% BSA	100% BSA
23	0.609	0.487	1.001	80% BSA + 20% HSA	80% BSA + 20% HSA
24	0.590	0.412	1.010	60% BSA + 40% HSA	60% BSA + 40% HSA

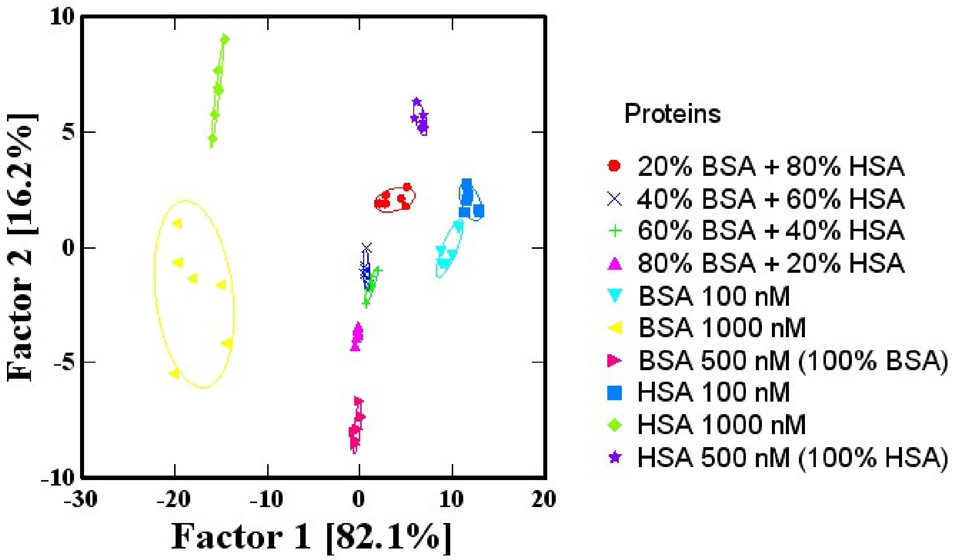


Figure S6. Canonical score plot for the three-channel patterns as obtained from LDA for BSA and HSA at different concentrations (100, 500 and 1000nM) and mixtures of BSA and HSA at different molar ratios (total protein concentration: 500 nM).

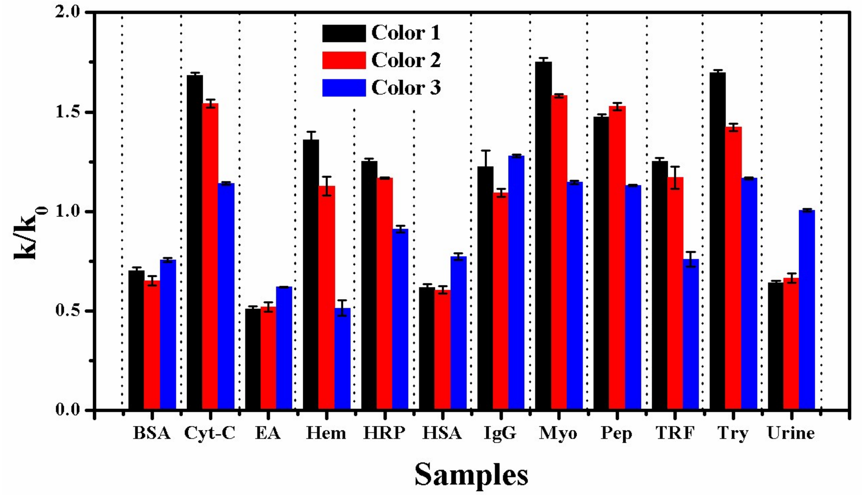


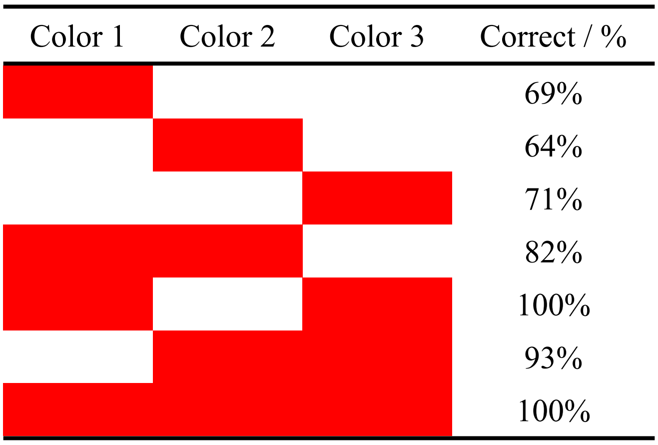
Figure S7. Fingerprints of proteins at the 500 nM level in the presence of human urine based on the patterns of the corresponding values of k/k_0 obtained from the triple channel colorimetric sensor array.

Table S9. Training matrix of the response patterns against proteins at the 500 nM level in the presence of human urine

Proteins	Color 1	Color 2	Color 3
BSA	0.669	0.627	0.736
BSA	0.702	0.635	0.753
BSA	0.706	0.638	0.758
BSA	0.706	0.658	0.760
BSA	0.707	0.658	0.760
BSA	0.718	0.689	0.763
Cyt-C	1.670	1.518	1.131
Cyt-C	1.675	1.526	1.133
Cyt-C	1.677	1.536	1.140
Cyt-C	1.680	1.541	1.143
Cyt-C	1.694	1.556	1.145
Cyt-C	1.708	1.570	1.147
EA	0.491	0.493	0.618
EA	0.506	0.497	0.618
EA	0.509	0.507	0.619
EA	0.509	0.536	0.619
EA	0.522	0.540	0.622
EA	0.524	0.544	0.623
Hem	1.282	1.054	0.450
Hem	1.344	1.093	0.499
Hem	1.356	1.118	0.507
Hem	1.382	1.153	0.534
Hem	1.386	1.164	0.549
Hem	1.399	1.175	0.555
HRP	1.228	1.162	0.887
HRP	1.250	1.165	0.893
HRP	1.253	1.166	0.921
HRP	1.256	1.168	0.922
HRP	1.259	1.171	0.925
HRP	1.262	1.174	0.927
HSA	0.592	0.577	0.742

HSA	0.601	0.588	0.773
HSA	0.620	0.612	0.775
HSA	0.627	0.619	0.775
HSA	0.630	0.619	0.785
HSA	0.631	0.623	0.787
IgG	1.140	1.069	1.268
IgG	1.154	1.082	1.273
IgG	1.180	1.087	1.276
IgG	1.270	1.094	1.279
IgG	1.285	1.108	1.285
IgG	1.330	1.125	1.286
Myo	1.732	1.569	1.139
Myo	1.734	1.573	1.140
Myo	1.735	1.582	1.141
Myo	1.758	1.586	1.147
Myo	1.770	1.587	1.152
Myo	1.772	1.588	1.158
Pep	1.451	1.501	1.127
Pep	1.472	1.509	1.128
Pep	1.472	1.532	1.128
Pep	1.473	1.535	1.129
Pep	1.475	1.537	1.131
Pep	1.495	1.550	1.136
TRF	1.226	1.118	0.729
TRF	1.236	1.126	0.729
TRF	1.254	1.147	0.730
TRF	1.260	1.148	0.768
TRF	1.268	1.239	0.801
TRF	1.269	1.242	0.802
Try	1.681	1.393	1.160
Try	1.685	1.417	1.164
Try	1.686	1.420	1.167
Try	1.705	1.432	1.167
Try	1.707	1.434	1.168
Try	1.714	1.447	1.174

Urine	0.633	0.663	0.995
Urine	0.634	0.684	1.002
Urine	0.651	0.680	1.002
Urine	0.646	0.684	1.006
Urine	0.647	0.637	1.008
Urine	0.641	0.635	1.019



Color 1	Color 2	Color 3	Correct / %
🟥			69%
	🟥		64%
		🟥	71%
🟥	🟥		82%
🟥		🟥	100%
	🟥	🟥	93%
🟥	🟥	🟥	100%

Figure S8. Jackknifed classification matrix obtained using LDA for sensing channels for the 11 proteins in the presence of human urine.

Table S10. LDA classification accuracy of the target protein at 500 nM in the presence of human urine using the triple channel colorimetric sensor array. The values are taken from the Jackknifed classification matrix based on raw data listed in **Table S11**

	Color 1	Color 2	Color 3	Color 1 and 2	Color 1 and 3	Color 2 and 3	Triple Channels
BSA	83	33	33	100	100	100	100
Cyt-C	67	50	17	100	100	50	100
EA	100	100	100	100	100	100	100
HRP	17	67	100	50	100	100	100
HSA	67	100	83	100	100	100	100
Hem	83	17	100	100	100	100	100
IgG	50	83	100	67	100	100	100
Myo	100	100	33	100	100	100	100
Pep	100	50	83	100	100	67	100
TRF	17	0	0	0	100	100	100
Try	50	100	100	100	100	100	100
Urine	100	67	100	67	100	100	100
Total	69	64	71	82	100	93	100

Table S11 Identification of unknown protein samples in the presence of human urine

	Color 1	Color 2	Color 3	Identification	Verification
1	1.293	1.287	0.815	TRF	TRF
2	1.194	1.181	0.882	HRP	HRP
3	1.731	1.661	1.166	Myo	Myo
4	0.561	0.482	0.641	EA	EA
5	1.414	1.456	1.105	Pep	Pep
6	0.581	0.562	0.732	HSA	HSA
7	0.693	0.625	0.687	BSA	BSA
8	1.203	1.042	0.340	Hem	Hem
9	0.722	0.697	0.732	BSA	BSA
10	1.270	1.208	0.885	HRP	HRP
11	1.711	1.571	1.184	Cyt-C	Cyt-C
12	1.270	1.115	0.706	TRF	TRF
13	1.095	1.044	1.242	IgG	IgG
14	1.658	1.504	1.125	Cyt-C	Cyt-C
15	1.120	1.057	1.253	IgG	IgG
16	1.272	1.212	0.927	HRP	HRP
17	1.791	1.613	1.158	Myo	Myo
18	1.167	1.179	0.855	HRP	HRP
19	0.567	0.547	0.655	EA	EA
20	1.443	1.563	1.139	Pep	Pep
21	0.665	0.640	0.816	HSA	HSA
22	0.759	0.705	0.789	BSA	BSA
23	1.082	1.019	0.336	Hem	Hem
24	1.716	1.453	1.175	Try	Try
25	1.446	1.053	0.563	Hem	Hem
26	1.733	1.454	1.175	Try	Try
27	0.755	0.699	0.769	BSA	BSA
28	1.506	1.564	1.146	Pep	Pep
29	0.660	0.634	0.814	HSA	HSA
30	1.390	1.455	1.093	Pep	Pep
31	0.484	0.473	0.611	EA	EA
32	1.780	1.560	1.129	Myo	Myo

33	1.301	1.289	0.824	TRF	TRF
34	1.723	1.595	1.185	Cyt-C	Cyt-C
35	1.368	1.174	1.291	IgG	IgG
36	1.652	1.369	1.148	Try	Try
37	1.453	1.198	0.579	Hem	Hem
38	1.666	1.390	1.152	Try	Try
39	1.124	1.067	1.289	IgG	IgG
40	1.710	1.517	1.130	Cyt-C	Cyt-C
41	1.215	1.111	0.698	TRF	TRF
42	1.803	1.634	1.159	Myo	Myo
43	0.480	0.473	0.600	EA	EA
44	0.588	0.567	0.737	HSA	HSA

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