Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Plasma-Assisted Quadruple-Channel Optosensing of Proteins and Cells with Mn-Doped ZnS Quantum Dots

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Experimental Section

Chemicals

All reagents used in this work were of analytical grade. Mercaptopropionic acid (MPA) (Aladin, Shanghai, China), $Zn(Ac)_2 \cdot 2H_2O$, $Mn(Ac)_2 \cdot 4H_2O$ and $Na_2S \cdot 9H_2O$ (Kelong Reagent Company, Chengdu, China) were used for the preparation of Mn-doped ZnS QDs. All proteins were purchased from Sigma-Aldrich. Ultrapure water (18.2 MΩ cm) was obtained from a water purification system (PCUJ-10, Chengdu Pure Technology Co., Chengdu, China).

Synthesis of mercaptopropionic acid (MPA)-capped Mn-doped ZnS QDs

MPA-capped Mn-doped ZnS QDs were prepared according to a previous publication.[S1] Briefly, to a three-necked flask, aqueous solutions of $ZnAc_2$ (5 mL, 0.1 M), $Mn(Ac)_2$ (0.2 mL, 0.1 M), and MPA (0.17 mL) were added and the final volume of the mixture was made to 50 mL with ultrapure water. The pH of the mixed solution was adjusted to 11.0 with 1 M NaOH. After removal of air by argon bubbling for 30 min at room temperature, 5 mL of 0.1 M Na_2S was quickly injected into the solution. The mixture was stirred for 20 min, and then the solution was aged at 50 °C under open air for 2 h to form MPA capped Mn-doped ZnS QDs. For purification, the obtained QDs were precipitated with ethanol, separated by centrifuging, washed with ethanol, and finally vacuum-dried.

Apparatus

The IF, FL, Ph and RLS signals were measured with an F-7000 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm). A 380 nm long-pass filter and a 300 nm long-pass filter were positioned in front of the emission window for measurement of FL (Mn-ZnS QDs) and IF (proteins), respectively. In this manner, interferences from such two bands were eliminated therefore. Mn-ZnS QD and proteins were both dissolved in PBS buffer (pH 7.4, 0.01 mM) before reaction. Mn-ZnS QDs (675 μL, 20 mg L^{-1}) and various amounts of the plasma treated proteins (each with 35 μL, 1 μM in a 12-well culture plate) were mixed thoroughly, and were left to stand for 10 min to measure the IF, FL, Ph and RLS signal intensity. All measurements were repeated to generate five replicates for each protein. Accordingly, for a given concentration, a 4 channels × 12 proteins × 5 replicates data matrix was generated and processed with classical linear discriminant analysis (LDA) in Matlab (The MathWorks Inc., USA).

Absorption spectra were recorded on a UV-1750 UV-vis spectrophotometer (Shimadzu, Japan). The powder X-ray diffraction (PXRD) patterns were obtained from an X'Pert Pro MPD (Philips, Netherlands) using Cu_{ka} radiation. TEM characterization was performed with a Tecnai G^2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., USA). Circular dichroism (CD) spectroscopy was performed in a 1 mm pathlength quartz cuvette using a spectropolarimeter (Chirascan; Applied Photophysics). Time-resolved fluorescence and phosphorescence decay by delay were performed on a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon Co. Ltd., France).The CD spectra were recorded for 180-260 nm at 25 °C. The Far-UV CD spectra of the samples were corrected by subtracting the corresponding spectra of buffers in the absence of proteins. The secondary structure parameters were computed using the "Dicroprot" software provided by the manufacturer.

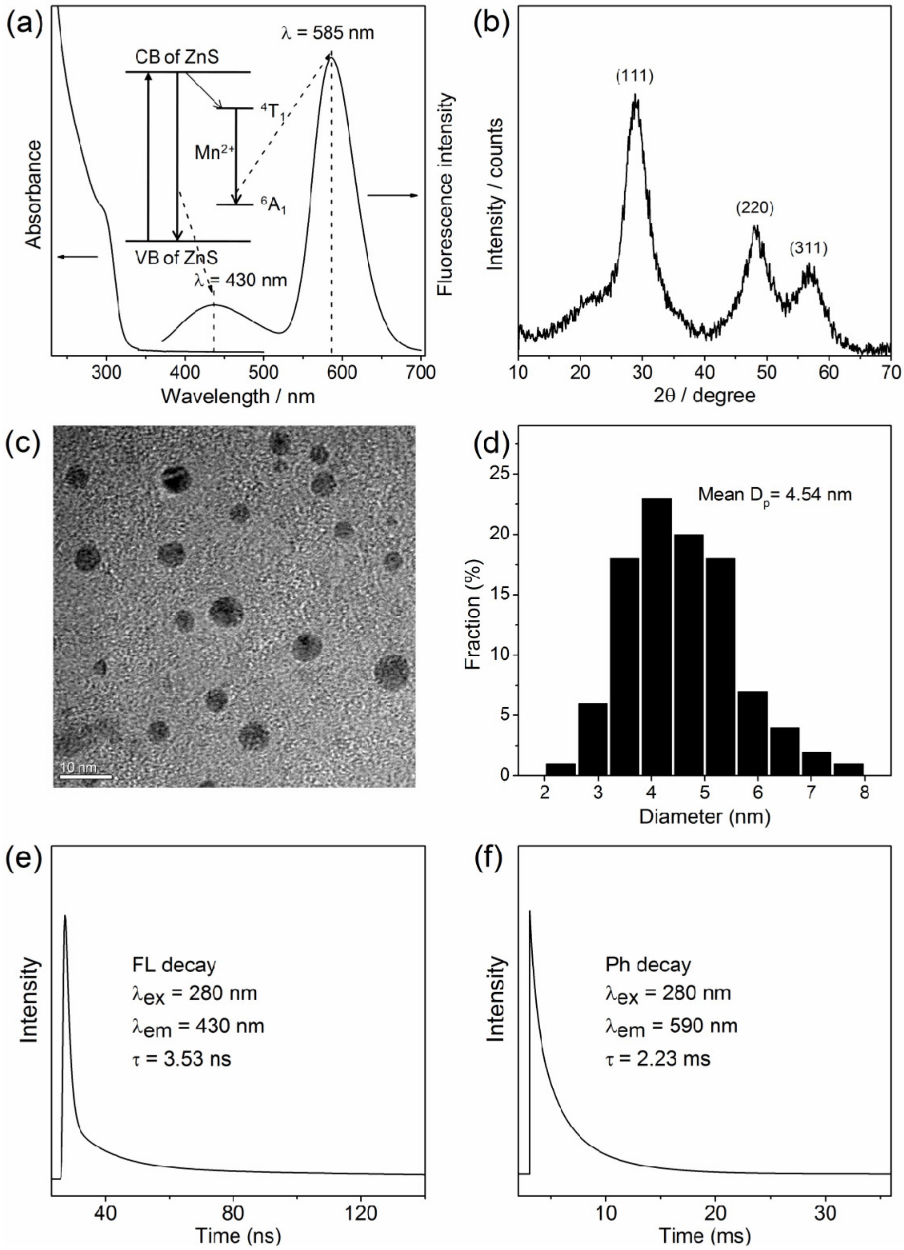

Fig. S1 Characterization of Mn-doped ZnS QDs. (a) UV-vis absorption spectra of Mn-doped ZnS QDs; (b) XRD pattern of Mn-doped ZnS QDs. It showed distinguishable (111), (220) and (311) planes of typical zinc blend structure; (c) TEM images of Mn-doped ZnS QDs; and (d) average diameter of Mn-doped ZnS QDs was 4.54 nm. Time-resolved fluorescence (e) and phosphorescence (f) decay of Mn-doped ZnS QDs.

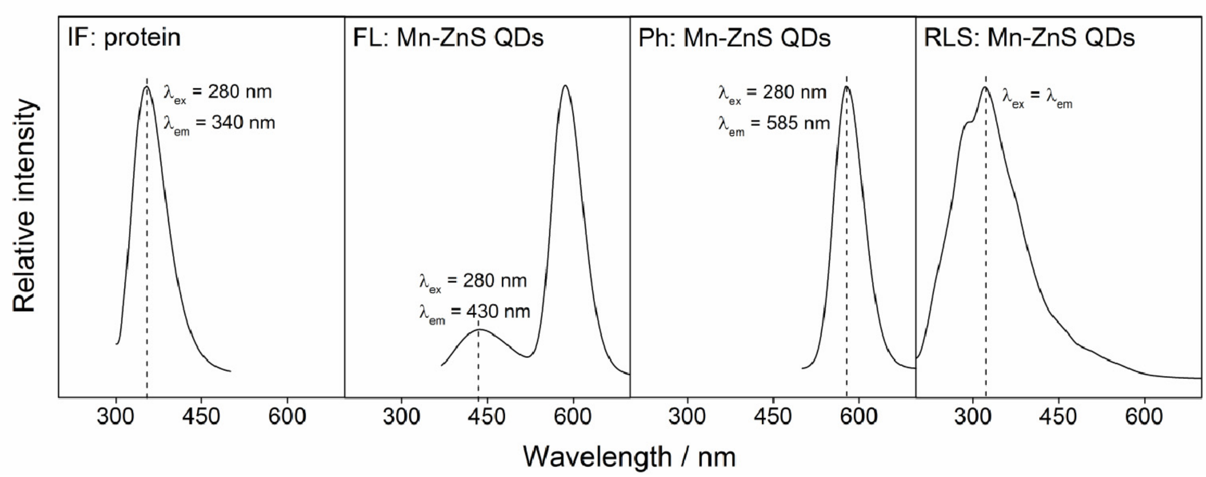

Fig. S2 The quadruple-channel optosensing mode. The IF, FL and Ph modes share the same excitation wavelength (λ_{ex} ~ 280 nm). Instrumental conditions: IF and FL mode: the Ex and Em spectral bandwidth: 10 nm; and the PMT voltage: -700 V; Ph mode: Ex spectral bandwidth: 10 nm; Em spectral bandwidth: 20 nm; and the PMT voltage: -700 V; RLS mode: Ex spectral bandwidth: 5 nm; Em spectral bandwidth: 10 nm; and the PMT voltage: -400 V.

Low-temperature DBD plasma for proteins treatment

A low-temperature dielectric barrier discharge (DBD) plasma was constructed and used for proteins treatment. As shown in Fig. S3, a cylindrical laboratory-built device consisting of a quartz tube (2.0 mm i.d. × 4.0 mm o.d. × 50 mm long) and two electrodes for discharge (Fig. S3). A tungsten (W) electrode (1.0 mm in diameter) was inserted into the tube as the inner electrode; another copper (Cu) electrode (0.5 mm in diameter) was tightly wrapped around the outer side of the quartz tube as the outer electrode. An AC ozone generation power supply (YG. BP105P, Electronic Equipment Factory of Guangzhou Salvage, Guangzhou, China; 6 cm long × 4 cm wide × 3 cm high, with a rated output of 4 kV, 20 kHz, and 12W at 220 V, 50 Hz input) was applied to the two electrodes of the DBD to provide the necessary voltage for generation of the DBD plasma. A transformer (TDGC2-1, Tianzheng Electronic Equipment Ltd. Co., Tianjin, China) was connected to the ozone generation power supply for adjusting the discharge power. Argon (Ar, 99.99%) (Qiaoyuan Gas Co. Ltd., Chengdu, China) was used as the discharge gas. During the discharge, a blue-violet plasma jet was generated at the end of the quartz tube. For proteins treatment, the distance between the quartz tube and the plate was set at 10 mm for both efficient contact with protein samples and well-retaining of the plasma jet.

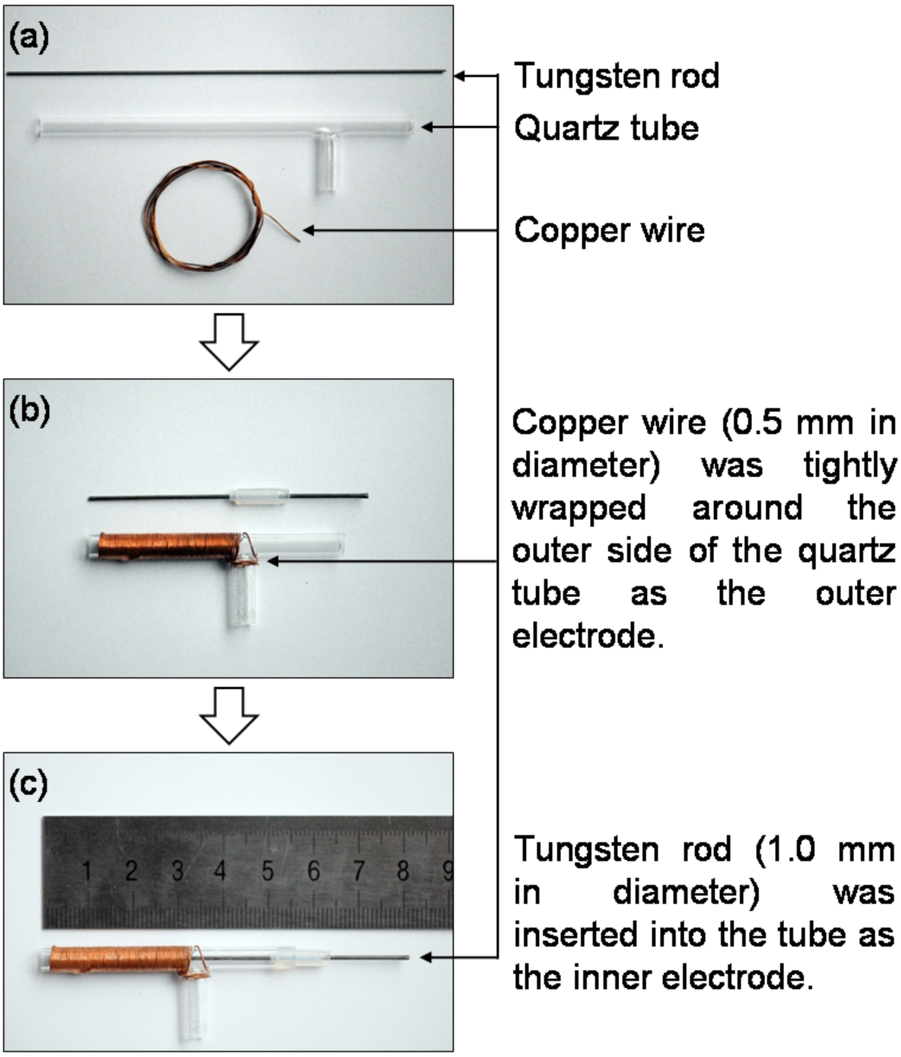

Fig. S3 The construction of the DBD device.

To achieve better discrimination resolution of the quadruple-channel optosensing device, the discharge conditions such as diacharge gas, input voltage and diacharge time should be optimized. In particular, the plasma gas plays an important role in the atomization, excitation and ionization of atoms/redicals. The DBD plasma is easily maintained without any modification when using N_2, Ar or He as the plasma gas. Argon showed the best performance at a flow rate of 600 mL min^{-1} for the optosensing device. An appropriate discharge voltage should be applied between the two electrodes. The discharge voltage was controlled by adjusting the input voltage of the AC ozone generation power supply through a transformer. The maximum signal appeared when the input voltage ranged from 130 to 170 V. As shown in Fig. S4, an input voltage of 150 V AC for 5min treatment was the best choice.

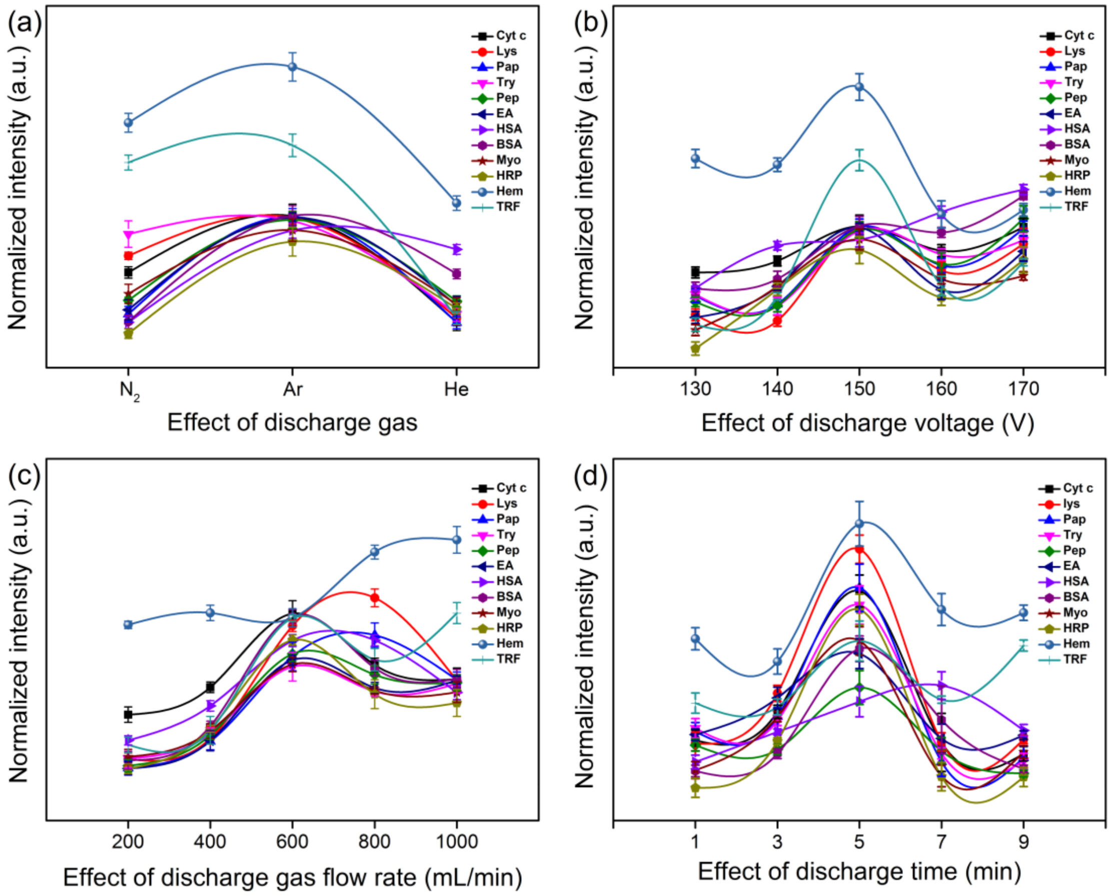

Fig. S4 The discharge conditions were optimized by detecting IF, FL, Ph and RLS response (i.e., ($\Delta_{IF}^2 + \Delta_{FL}^2 + \Delta_{Ph}^2 + \Delta_{RLS}^2)^{1/2}$).

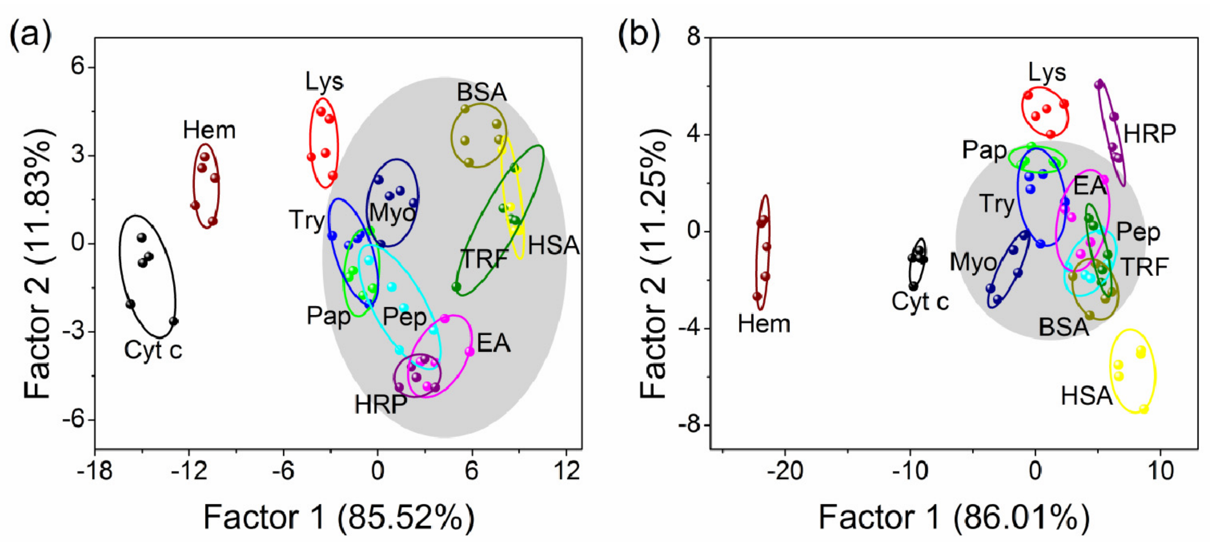

Fig. S5 Canonical score plot for the patterns (from the previously reported triple-channel optosensing device [S1] obtained from LDA against twelve proteins at concentration level of 50 nM without (a) and with (b) the plasma treatment, with 95% confidence ellipses.

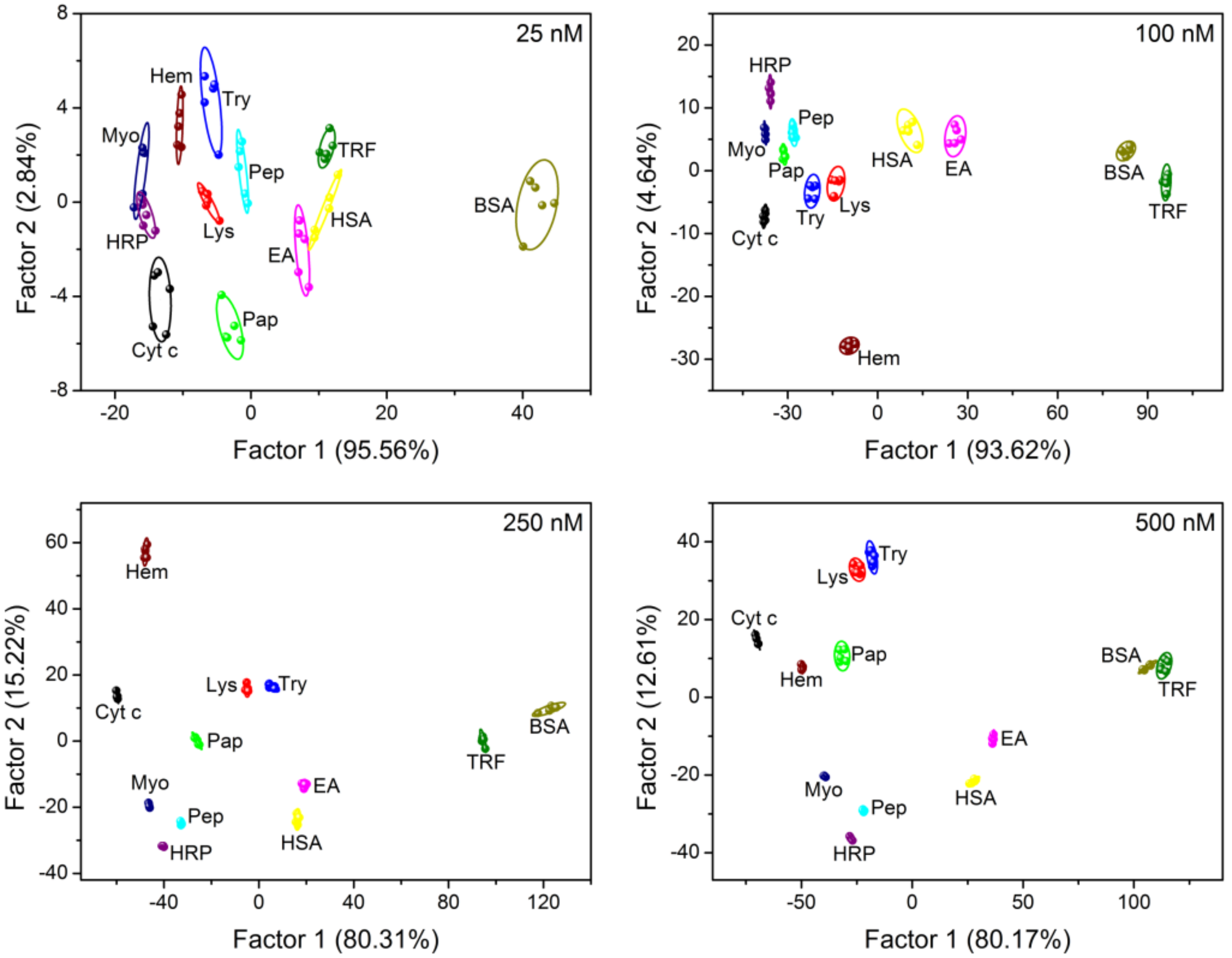

Fig. S6 Array-based sensing of the twelve proteins at different concentration. Canonical score plot for for the IF, FL, Ph and RLS patterns obtained from the LDA against different concentrations of proteins (25 nM, 100 nM, 250 nM and 500 nM).

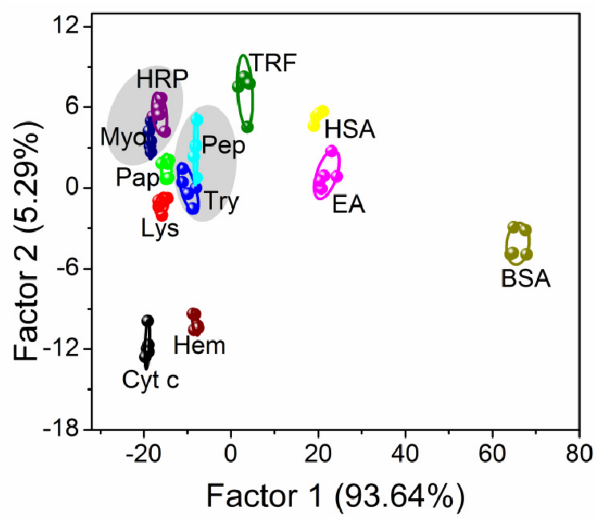

Fig. S7 Canonical score plot for the FL, Ph, RLS and IF patterns obtained from LDA against twelve proteins without the plasma treatment.

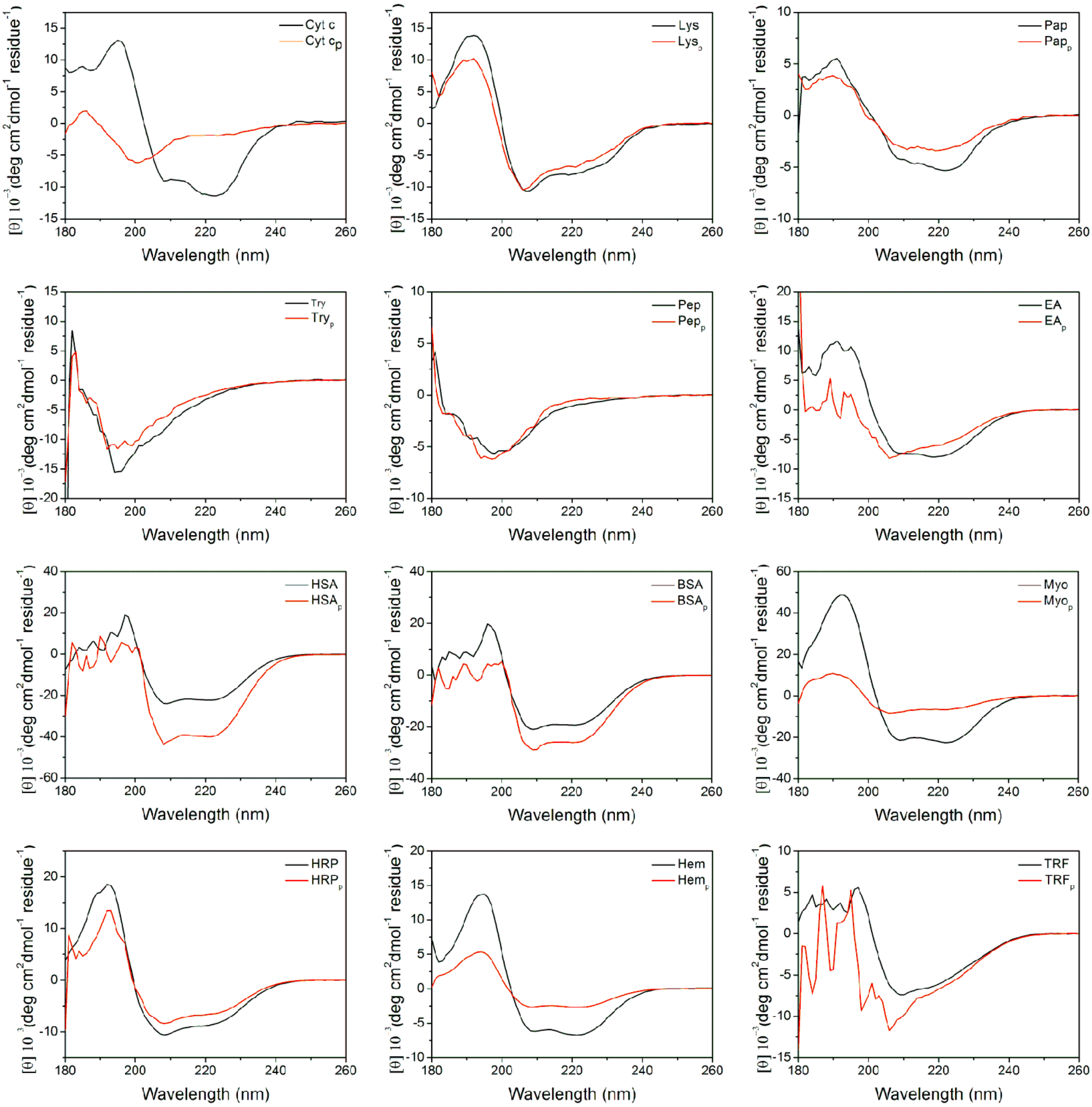

Fig. S8 Circular dichroism (CD) spectra of the twelve treated and untreated proteins. The subscript “p” represents the plasma-treated proteins.

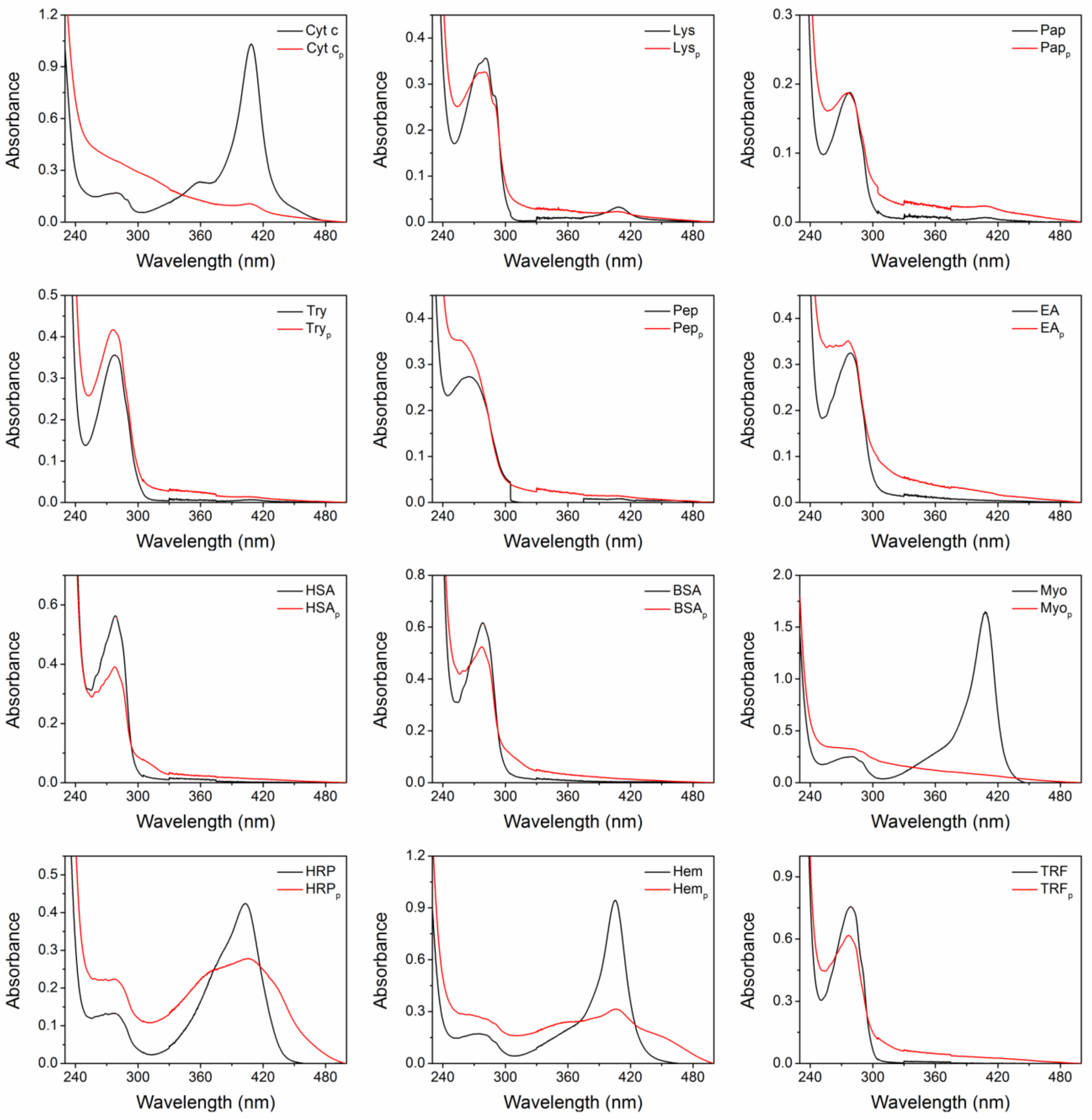

Fig. S9 UV-vis spectra of the twelve treated and untreated proteins. The subscript "p" represents the plasma treated proteins.

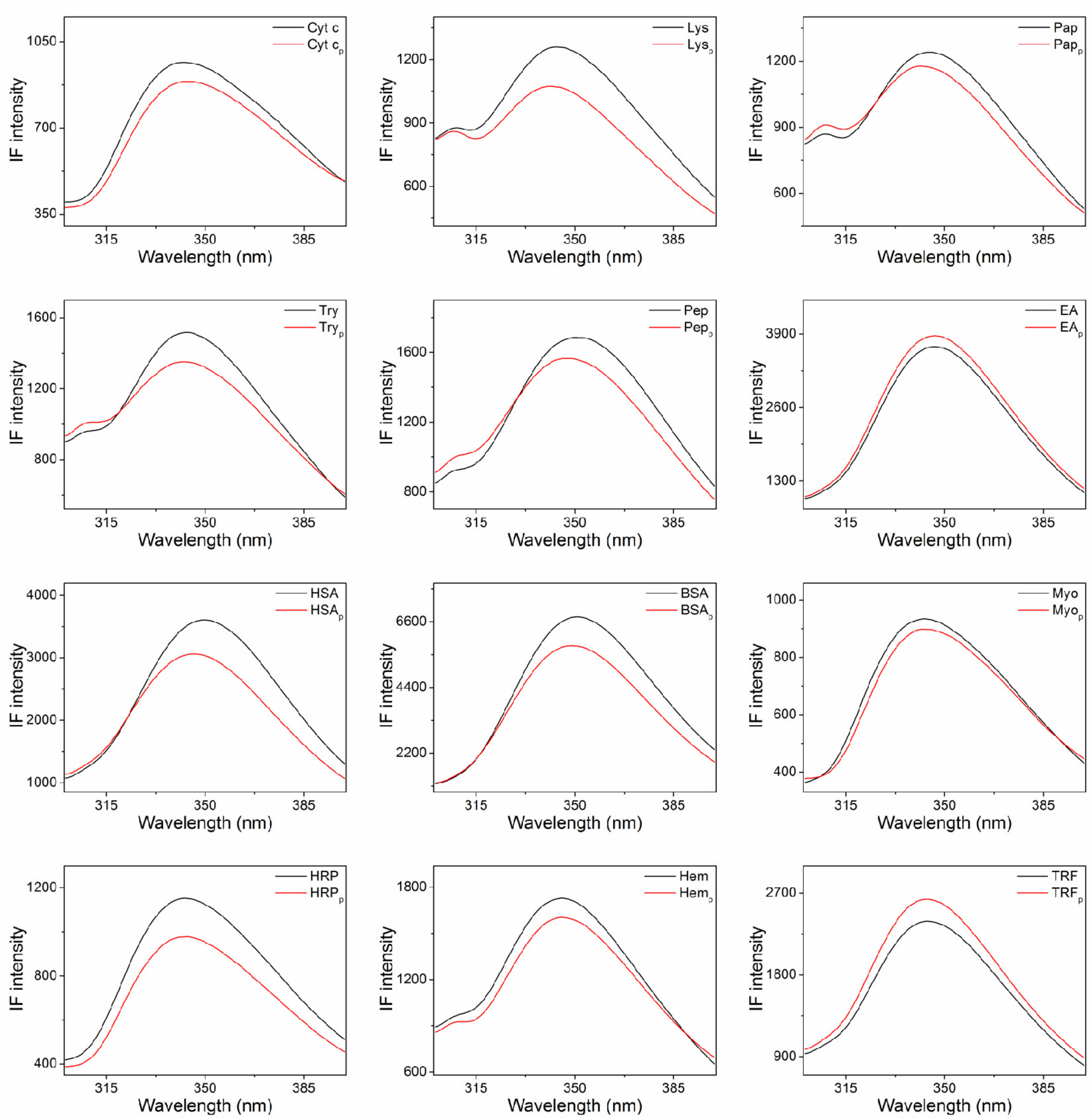

Fig. S10 IF spectra of the twelve treated and untreated proteins. The subscript "p" represents plasma treated proteins.

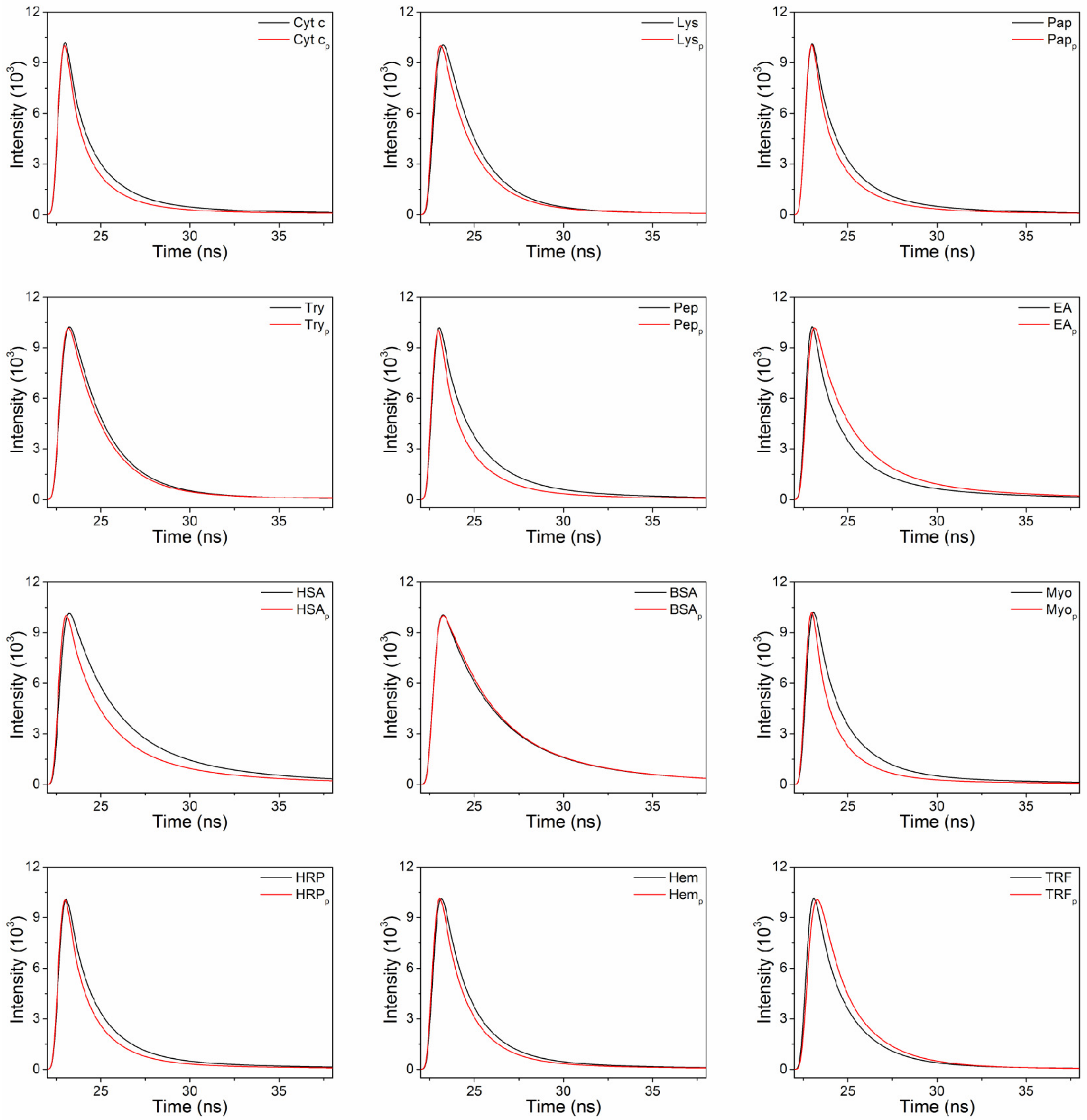

Fig. S11 Time-resolved fluorescence decay by delay of the twelve treated and untreated proteins. The subscript “p” represents the plasma treated proteins. Instrumental conditions: λ_{ex} = 280 nm; and λ_{em} = 335 nm.

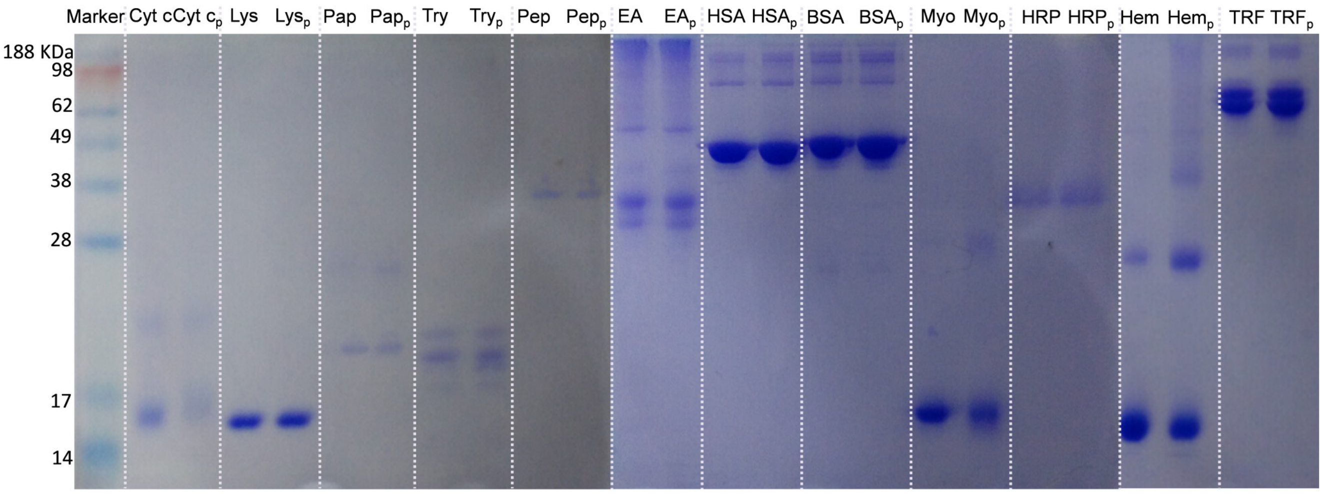

Fig. S12 SDS-PAGE of the untreated and the plasma treated proteins. The SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed in a vertical discontinuous gel system, including separating (15%, m/v) and stacking (5%, m/v) gels. Gel stock solution (30%, w/v) contained 29.2 g of acrylamide and 0.8 g of Bis, which were dissolved in 100 mL of H_2O and filtrated. The stacking gel (5%, m/v) solution was prepared by mixing 0.83 mL of gel stock solution, 0.63 mL of Tris-HCl (1.0 M, pH 6.8), 50 μL of SDS (10%, w/v), 50 μL of $(NH_4)_2S_2O_8$ (10%, w/v), and 5 μL of TEMED and then diluting the mixture to 5.0 mL; 2.5 mL of gel stock solution was mixed with 1.3 mL of Tris-HCl (1.5 M, pH 8.8), 1.1 mL of ultrapure water, 50 μL of SDS (10%, w/v), 50 μL of $(NH_4)_2S_2O_8$ (10%, w/v), and 2 μL of TEMED to prepare the separating gel solution (15%, m/v). The voltage was set at 120 V when the protein sample was in the stacking gel, and tuned to 150 V after it entered into the separating one. The loading volume for each channel was 10 μL.

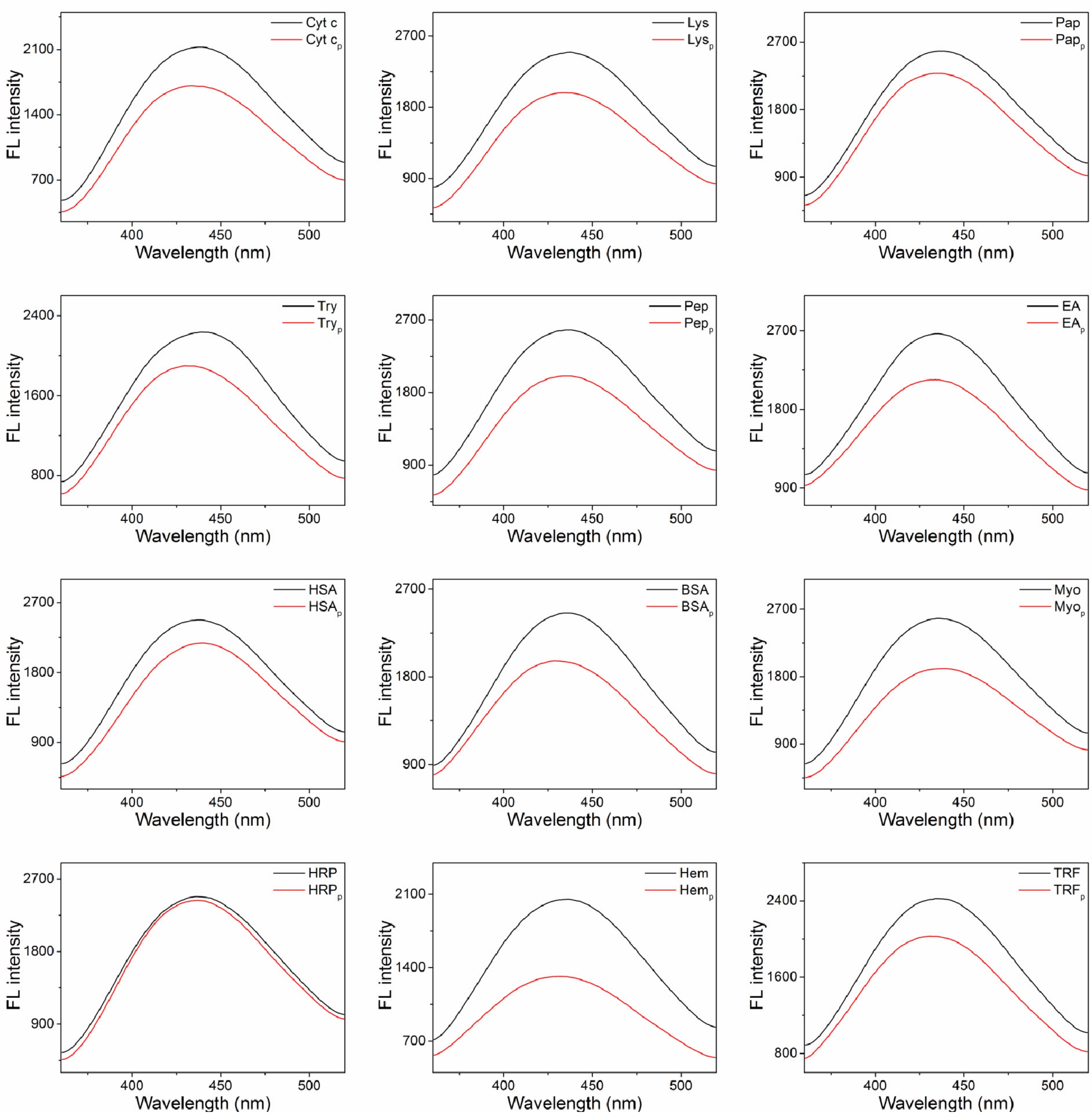

Fig. S13 FL spectra of Mn-ZnS QDs upon incubation with plasma treated proteins and untreated proteins. The subscript "p" represents the plasma treated proteins.

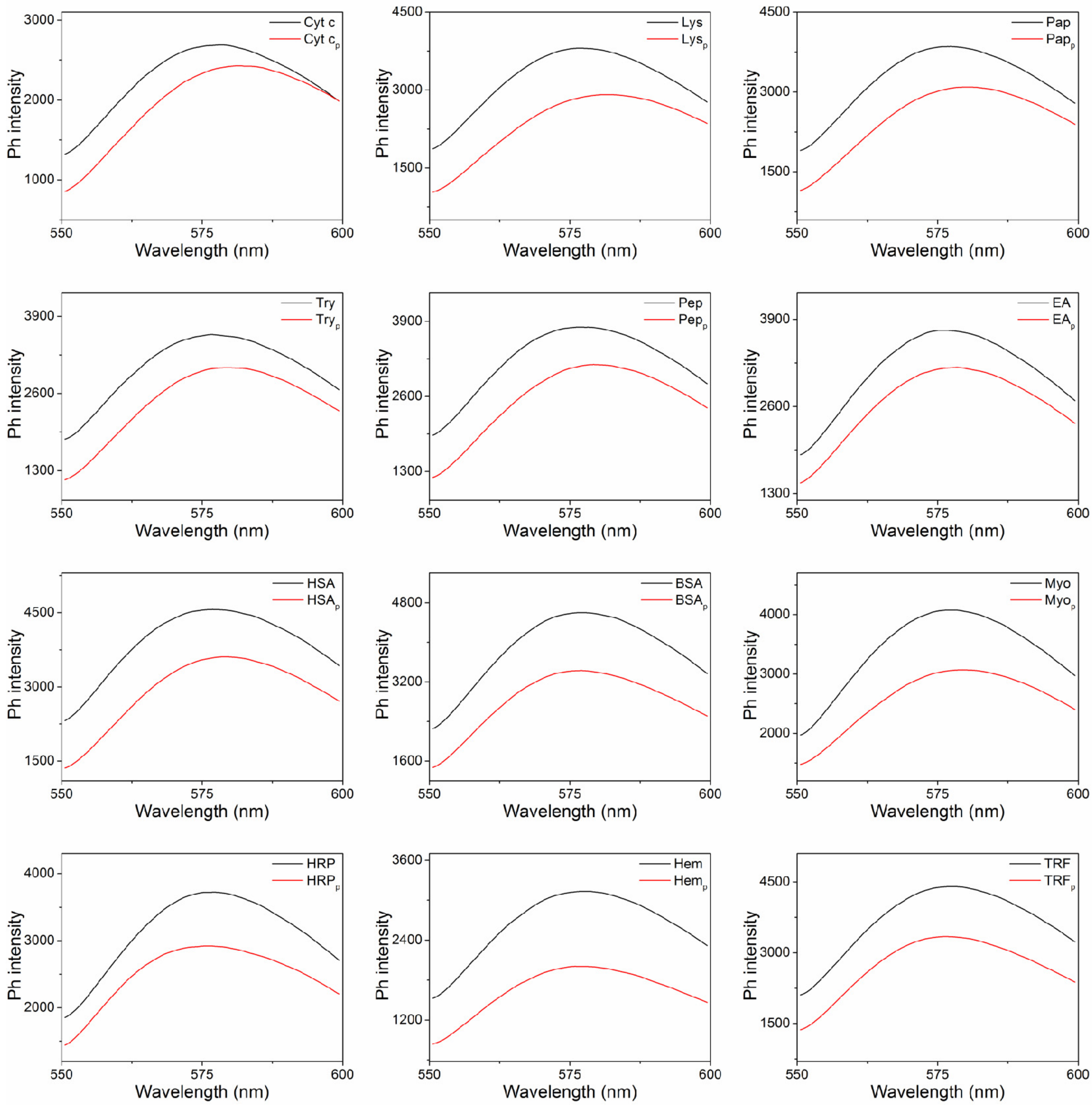

Fig. S14 Ph spectra of Mn-ZnS QDs upon incubation with plasma treated proteins and untreated proteins. The subscript “p” represents the plasma treated proteins.

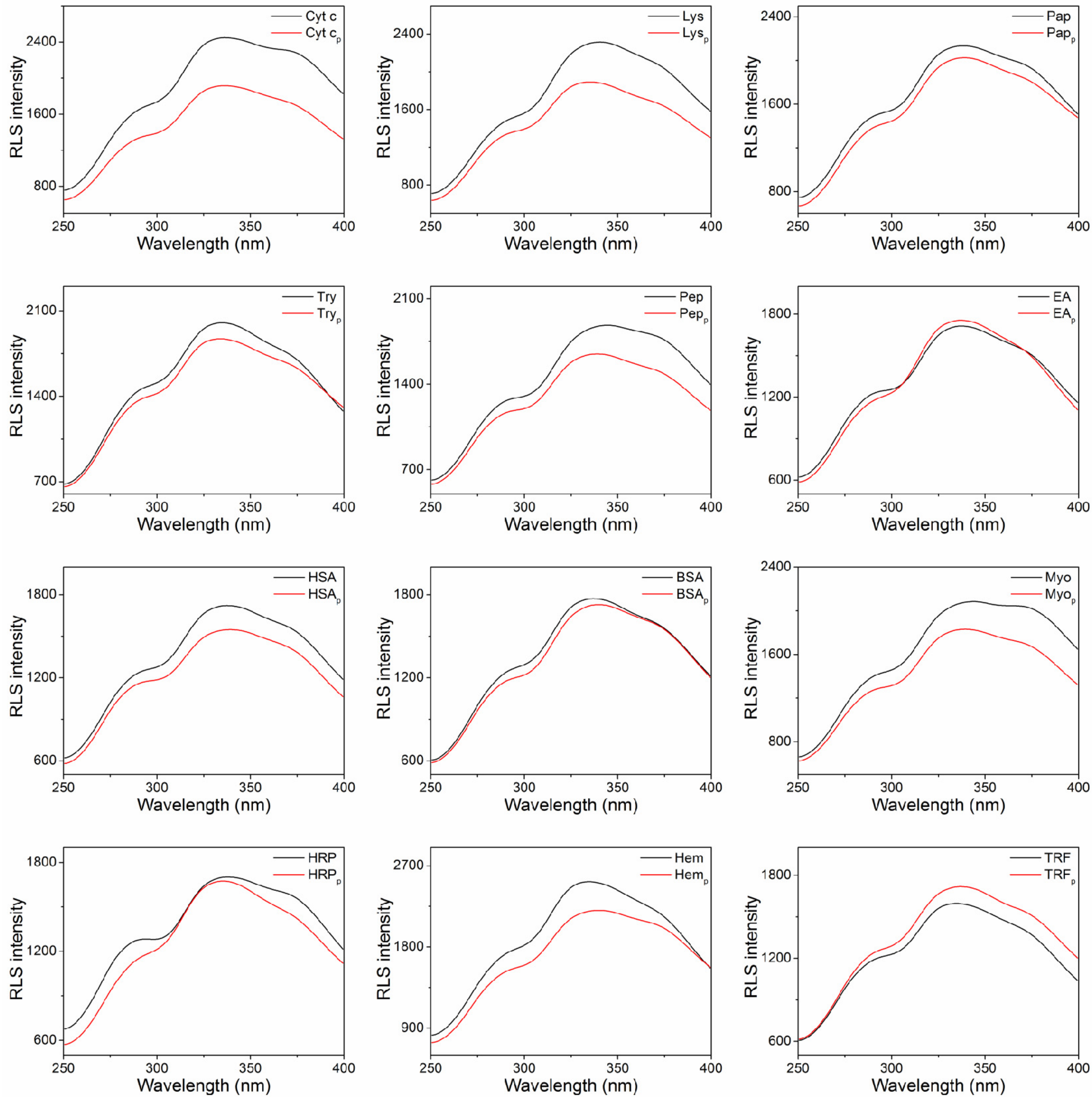

Fig. S15 RLS spectra of Mn-ZnS QDs upon incubation with plasma treated proteins and untreated proteins. The subscript "p" represents the plasma treated proteins.

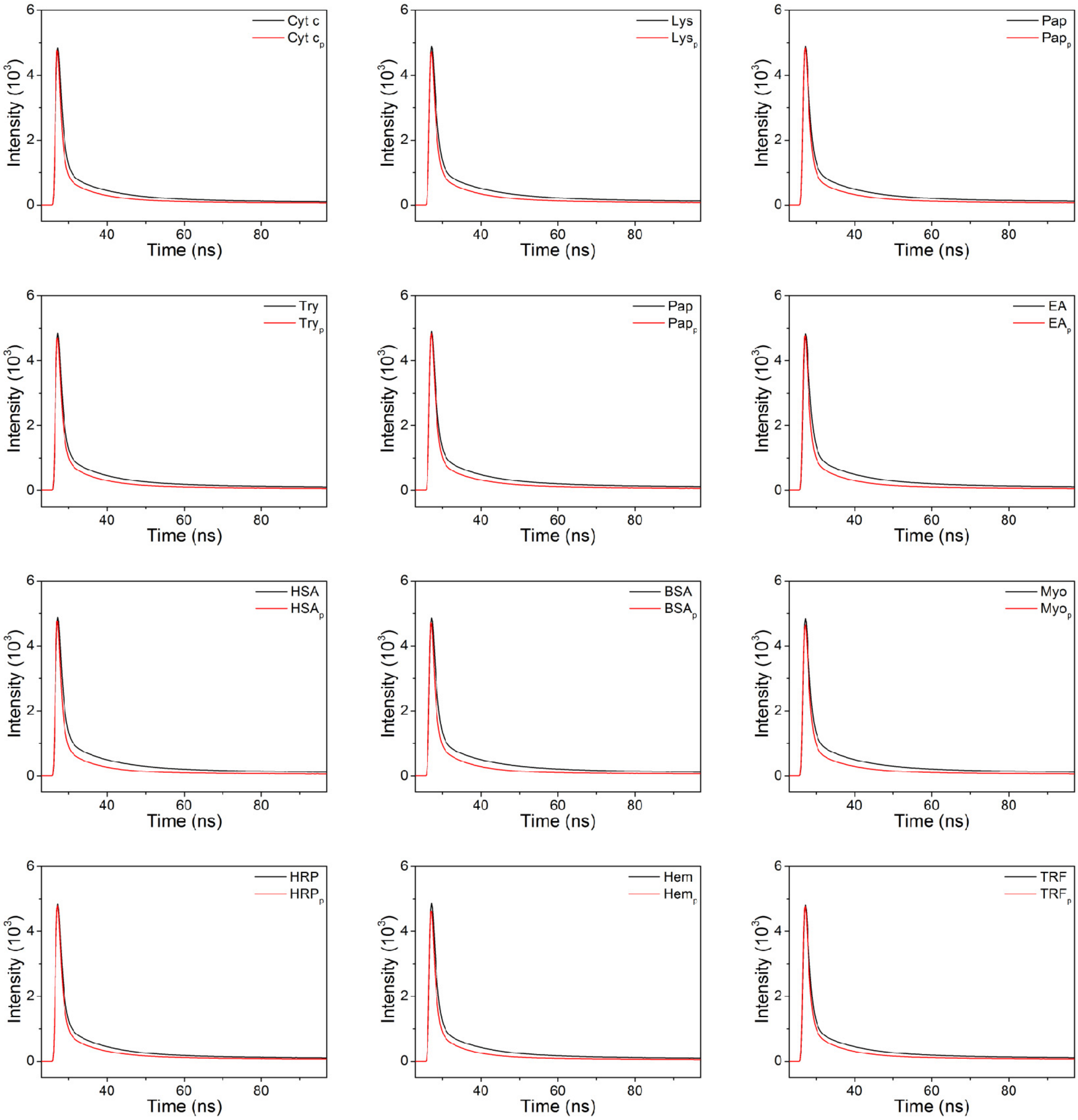

Fig. S16 Time-resolved fluorescence decay of Mn-ZnS QDs upon incubation with plasma treated proteins and untreated proteins (λ_{ex} = 280 nm; λ_{em} = 430 nm.). The subscript “p” represents the plasma treated proteins.

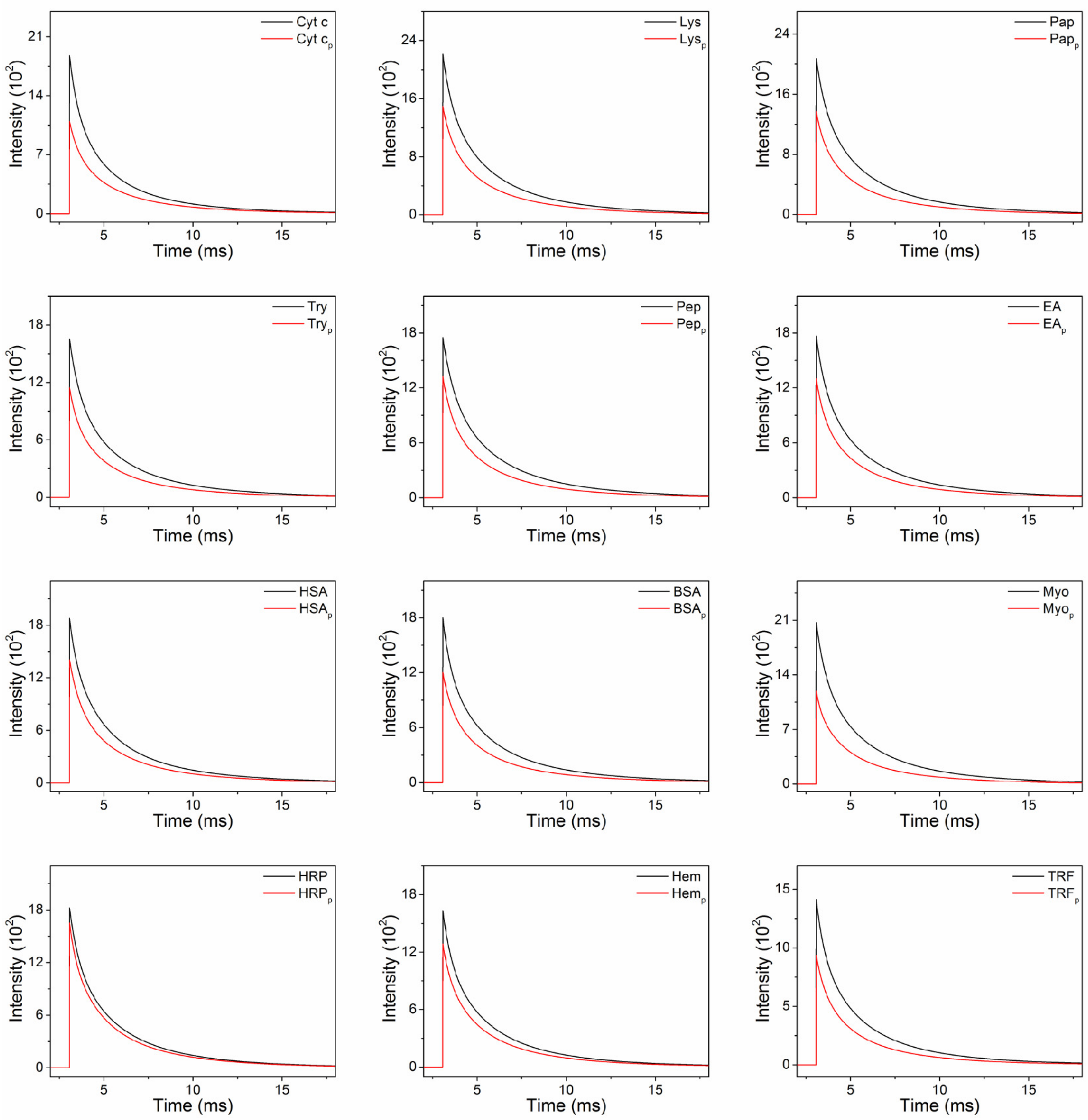

Fig. S17 Time-resolved phosphorescence decay of Mn-ZnS QDs upon incubation with plasma treated proteins and untreated proteins (λ_{ex} = 280 nm; λ_{em} = 590 nm.). The subscript "p" represents the plasma treated proteins.

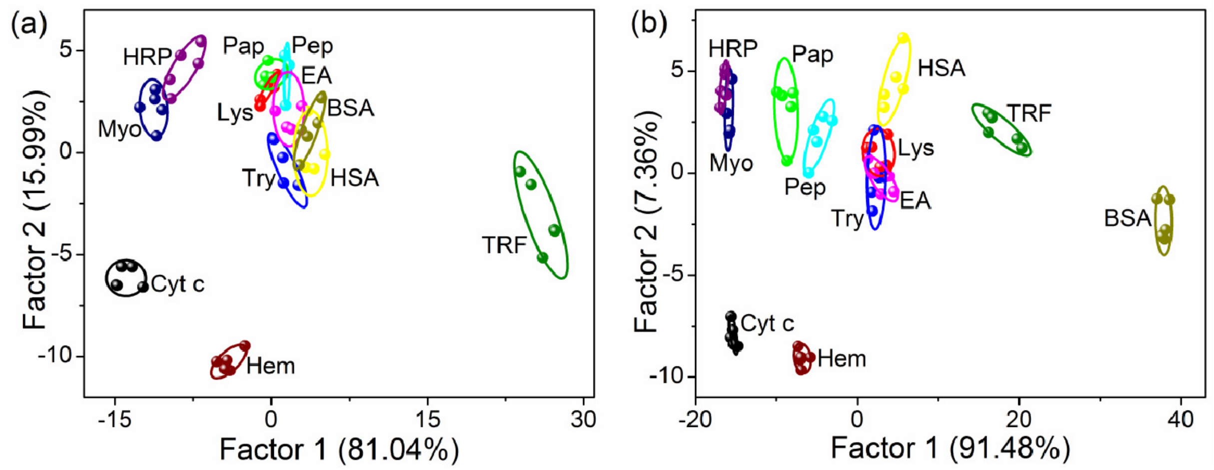

Fig. S18 Canonical score plot for the IF, FL, Ph and RLS patterns obtained from the LDA against different protein denaturation methods for 5 min: (a) UV irradiation from a low-pressure Hg vapor UV lamp (15 W), and (b) heating (80 °C), with 95% confidence ellipses.

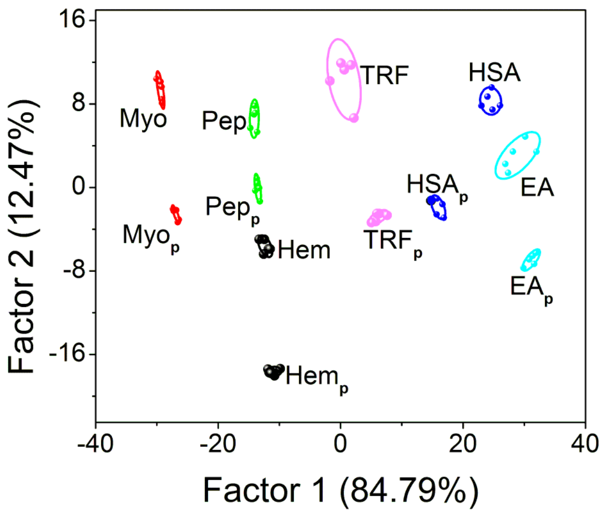

Fig. S19 Canonical score plot for the IF, FL, Ph and RLS patterns obtained from the LDA against native proteins and the plasma denatured proteins, with 95% confidence ellipses. The subscript "p" represents the plasma treated proteins.

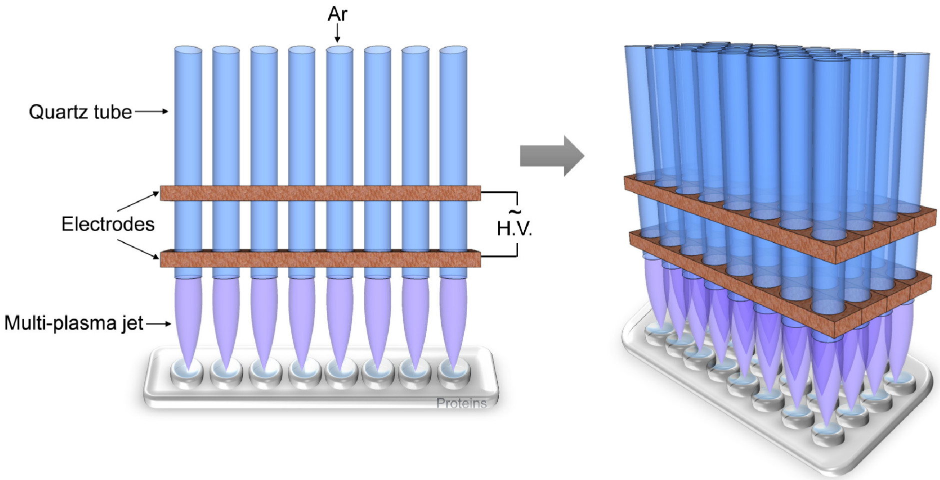

Fig. S20 Schematic illustration of the use of DBD plasma array for protein treatment.

Sample analysis

Urine sample was collected from a healthy volunteer in the morning. To avoid the RLS, FL and IF background of the pure urine sample, the collected sample was centrifuged for 5 min at 10000 rpm and diluted 100-fold before analysis. For multidimensional sensing of proteins in human urine sample, all solutions were mixed thoroughly for 10 min before the measurement of FL, Ph, RLS and IF intensity. The protein-spiked samples were made by adding 990 μL of centrifuged urine spiked with varied amounts of proteins (10 μL, 100 μM and 20 μL, 100 μM), then treated with the plasma.

The human serum sample was collected from a local hospital and diluted 100-fold before analysis. The multidimensional sensing of proteins in the human serum sample was the same as that in the urine sample.

All the cells were grown in cell culture media and incubated at 37 ºC in a 5% CO_2/95% air humidified incubator. After receiving the cells, they were centrifuged to remove the culture media and washed with cold PBS buffer (pH 7.4) for three times. After re-dispersing in PBS buffer (1 mL), cells were sonicated for 10 min and then centrifuged again. The supernatant was collected and subjected to the above analytical procedure.

Table S1 Origin and nature of mammalian cell lines used in this study.

	Origin	Cell lines	Status
Human cell lines	umbilical cord	HUVEC	normal
	breast	MCF-7	cancerous
	lung	H446	cancerous
	ovary	SKOV-3	cancerous
	lymph	OCI-LY1	cancerous
	lymph	SU-DHL-6	cancerous
Mouse cell lines	breast	4T1	metastatic

Jack-knifed classification matrix obtained using LDA from the quadruple-channel optosensing device (IF, FL, Ph and RLS) for twelve proteins with (Table S2) and without (Table S3) the plasma treatment; and triple-channel optosensing device (FL, Ph and RLS) for twelve proteins with (Table S4) and without (Table S5) the plasma treatment.

Table S2	Cyt c	Lys	Pap	Try	Pep	EA	HSA	BSA	Myo	HRP	Hem	TRF
Cyt c	5	0	0	0	0	0	0	0	0	0	0	0
Lys	0	5	0	0	0	0	0	0	0	0	0	0
Pap	0	0	5	0	0	0	0	0	0	0	0	0
Try	0	0	0	5	0	0	0	0	0	0	0	0
Pep	0	0	0	0	5	0	0	0	0	0	0	0
EA	0	0	0	0	0	5	0	0	0	0	0	0
HSA	0	0	0	0	0	0	5	0	0	0	0	0
BSA	0	0	0	0	0	0	0	5	0	0	0	0
Myo	0	0	0	0	0	0	0	0	5	0	0	0
HRP	0	0	0	0	0	0	0	0	0	5	0	0
Hem	0	0	0	0	0	0	0	0	0	0	5	0
TRF	0	0	0	0	0	0	0	0	0	0	0	5
Total N	5	5	5	5	5	5	5	5	5	5	5	5
N correct	5	5	5	5	5	5	5	5	5	5	5	5
Proportion	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
N = 60			N correct = 60					Proportion correct = 1.000				

Table S3	Cyt c	Lys	Pap	Try	Pep	EA	HSA	BSA	Myo	HRP	Hem	TRF
Cyt c	5	0	0	0	0	0	0	0	0	0	0	0
Lys	0	5	0	0	0	0	0	0	0	0	0	0
Pap	0	0	5	0	0	0	0	0	0	0	0	0
Try	0	0	0	4	1	0	0	0	0	0	0	0
Pep	0	0	0	1	4	0	0	0	0	0	0	0
EA	0	0	0	0	0	5	0	0	0	0	0	0
HSA	0	0	0	0	0	0	5	0	0	0	0	0
BSA	0	0	0	0	0	0	0	5	0	0	0	0
Myo	0	0	0	0	0	0	0	0	5	0	0	0
HRP	0	0	0	0	0	0	0	0	0	5	0	0
Hem	0	0	0	0	0	0	0	0	0	0	5	0
TRF	0	0	0	0	0	0	0	0	0	0	0	5
Total N	5	5	5	5	5	5	5	5	5	5	5	5
N correct	5	5	5	4	4	5	5	5	5	5	5	5
Proportion	1.00	1.00	1.00	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00
N = 60			N correct = 58					Proportion correct = 0.996				

Table S4	Cyt c	Lys	Pap	Try	Pep	EA	HSA	BSA	Myo	HRP	Hem	TRF
Cyt c	5	0	0	0	0	0	0	0	0	0	0	0
Lys	0	5	0	0	0	0	0	0	0	0	0	0
Pap	0	0	3	2	0	0	0	0	0	0	0	0
Try	0	0	2	3	0	0	0	0	0	0	0	0
Pep	0	0	0	0	3	2	0	0	0	0	0	0
EA	0	0	0	0	3	1	0	0	0	1	0	0
HSA	0	0	0	0	0	0	5	0	0	0	0	0
BSA	0	0	0	0	0	0	0	3	0	0	0	2
Myo	0	0	0	1	0	0	0	0	4	0	0	0
HRP	0	0	0	0	0	0	0	0	0	5	0	0
Hem	0	0	0	0	0	0	0	0	0	0	5	0

TRF	0	0	0	0	0	0	0	2	0	0	0	3
Total N	5	5	5	6	6	3	5	5	4	6	5	5
N correct	5	5	3	3	3	1	5	3	4	5	5	3
Proportion	1.00	1.00	0.60	0.50	0.50	0.33	1.00	0.60	1.00	0.83	1.00	0.60
N = 60			N correct = 45					Proportion correct = 0.750				

Table S5	Cyt c	Lys	Pap	Try	Pep	EA	HSA	BSA	Myo	HRP	Hem	TRF
Cyt c	5	0	0	0	0	0	0	0	0	0	0	0
Lys	0	5	0	0	0	0	0	0	0	0	0	0
Pap	0	0	2	1	1	0	0	0	1	0	0	0
Try	0	0	1	4	0	0	0	0	0	0	0	0
Pep	0	0	1	0	2	1	0	0	0	1	0	0
EA	0	0	0	0	0	3	0	0	0	2	0	0
HSA	0	0	0	0	0	0	2	1	0	0	0	2
BSA	0	0	0	0	0	0	0	5	0	0	0	0
Myo	0	0	1	0	0	0	0	0	4	0	0	0
HRP	0	0	0	0	0	1	0	0	0	4	0	0
Hem	0	0	0	0	0	0	0	0	0	0	5	0
TRF	0	0	0	0	0	1	3	0	0	0	0	1
Total N	5	5	5	5	3	6	5	6	5	7	5	3
N correct	5	5	2	4	2	3	2	5	4	4	5	1
Proportion	1.00	1.00	0.40	0.80	0.67	0.50	0.40	0.83	0.80	0.57	1.00	0.33
N = 60			N correct = 42					Proportion correct = 0.700				

Table S6 Detection and identification of 60 unknown protein samples at 50 nM.

Sample No.	Ph	RLS	FL	IF	Identification	Verification
1	3076	2220	2191	1113	Lys	Lys
2	3107	1900	2126	1328	Try	Try
3	3475	1746	1930	5772	BSA	BSA
4	3279	1940	2318	987	HRP	HRP
5	2854	1750	1925	947	Myo	Myo
6	2931	1887	1945	945	Myo	Myo
7	3306	1770	2051	2554	TRF	TRF
8	2532	2045	1701	865	Cyt c	Cyt c
9	2934	2065	2142	1214	Pap	Pap
10	3094	1655	2146	1594	Pep	Pep
11	1971	2318	1503	1662	Hem	Hem
12	3712	1492	1803	3035	HSA	HSA
13	3160	1779	2226	3772	EA	EA
14	2826	1771	1993	870	Myo	Myo
15	2981	2044	1969	1372	Try	Try
16	1886	2182	1439	1648	Hem	Hem
17	3345	1860	2057	2608	TRF	TRF
18	3019	1992	2140	1197	Pap	Pap
19	3125	1781	2123	3881	EA	EA

20	3356	1801	1973	5862	BSA	BSA
21	3209	1938	2308	961	HRP	HRP
22	2889	2109	2132	1277	Pap	Pap
23	2520	2119	1756	861	Cyt c	Cyt c
24	2968	2217	2154	1049	Lys	Lys
25	3062	1735	2154	3774	EA	EA
26	3136	1895	2398	1021	HRP	HRP
27	3219	1647	2121	1574	Pep	Pep
28	3592	1564	1884	3019	HSA	HSA
29	3008	1885	1887	877	Myo	Myo
30	3041	1754	2252	3799	EA	EA
31	1982	2374	1345	1652	Hem	Hem
32	3417	1746	1930	5974	BSA	BSA
33	3132	1611	2089	1618	Pep	Pep
34	3338	1812	1917	5882	BSA	BSA
35	2997	1984	2049	1368	Try	Try
36	3064	2201	2221	1110	Lys	Lys
37	3686	1542	1816	3010	HSA	HSA
38	3107	2066	2460	973	HRP	HRP
39	2899	1959	2149	1396	Try	Try
40	2001	2242	1374	1627	Hem	Hem
41	3258	1603	2101	1574	Pep	Pep
42	3144	2003	2319	999	HRP	HRP
43	3504	1558	1948	3063	HSA	HSA
44	3058	2273	2200	1020	Lys	Lys
45	3043	2057	2121	1223	Pap	Pap
46	2528	1993	1660	891	Cyt c	Cyt c
47	2797	1818	1875	919	Myo	Myo
48	3305	1820	2036	2653	TRF	TRF
49	2513	1912	1778	916	Cyt c	Cyt c
50	2978	1997	1993	1412	Try	Try
51	3293	1852	2018	2559	TRF	TRF
52	3045	2237	2089	1055	Lys	Lys
53	2498	2001	1724	902	Cyt c	Cyt c
54	3047	2050	2141	1218	Pap	Pap
55	3062	1780	2291	3757	EA	EA
56	3372	1886	2065	2476	TRF	TRF
57	3166	1572	2158	1616	Pep	Pep
58	3747	1495	1870	2954	HSA	HSA
59	3326	1843	1964	5812	BSA	BSA
60	2007	2279	1431	1635	Hem	Hem

Table S7 Training matrix of the response patterns against proteins at 3 different concentration levels based on the response from the quadruple-channel optical sensor.

Protein	25 nM				50 nM				100 nM			
	Ph	RLS	FL	IF	Ph	RLS	FL	IF	Ph	RLS	FL	IF
Cyt c	1981	1584	5155	390	1562	1892	5134	421	1489	2522	3610	474
Cyt c	2266	1557	5541	400	1657	1892	4959	405	1428	2505	3629	444
Cyt c	2006	1432	5746	388	1682	1785	4865	391	1517	2670	3903	459
Cyt c	1942	1404	5766	415	1650	1865	5048	385	1520	2489	3643	437
Cyt c	2303	1494	5799	425	1540	1809	4837	409	1490	2546	3925	466
Lys	2074	1646	5811	535	1805	1928	5359	536	2126	3218	4681	1952
Lys	2082	1700	5982	514	1931	2052	5352	526	1979	3080	4814	1987
Lys	1991	1735	6032	505	1897	1886	4859	537	1867	3191	4604	1849
Lys	1980	1699	5917	511	1948	1870	5210	523	1954	2963	4719	1817
Lys	1947	1734	5803	514	1898	1845	5199	527	1975	3070	4777	1884
Pap	2194	1435	6085	534	1790	1939	5224	586	1879	2590	5006	870
Pap	2085	1343	5726	535	1881	1860	5187	587	1885	2430	5034	846
Pap	2143	1339	5608	537	1860	1811	5165	566	1747	2470	4992	834
Pap	2157	1313	5825	567	1820	1830	5269	585	1748	2412	5011	891
Pap	2140	1357	5820	555	1647	1860	5189	575	1814	2411	5092	850
Try	2210	1963	5573	513	1956	1746	5368	667	1842	2838	4489	1430
Try	2204	1990	5651	533	1939	1778	5464	700	1816	3097	4446	1458
Try	2105	2039	5537	516	2003	1831	5402	670	1901	2857	4470	1489
Try	2119	1816	5772	539	2083	1723	5781	672	1747	2979	4426	1322
Try	1944	2018	5633	537	1999	1812	5802	660	1779	2899	4680	1374
Pep	2313	1678	5866	589	2068	1772	5608	873	1824	1895	4990	1167
Pep	2169	1656	6001	596	1967	1700	5885	891	1959	1843	4965	1130
Pep	2202	1754	6031	581	1901	1716	5651	874	1872	1853	5015	1085
Pep	2223	1802	5745	582	1975	1608	5888	879	1794	1833	4967	1096
Pep	2209	1821	5901	589	1973	1739	5754	873	1862	1749	5164	1113
EA	1967	1417	5598	710	1917	1980	5597	1991	1770	1874	5964	4508
EA	2077	1536	5636	706	1932	1826	5585	1978	1782	1902	5568	4646
EA	2124	1448	5769	691	1858	1837	5570	2044	1763	2053	5587	4379
EA	2257	1548	5478	692	1920	1820	5815	2076	1860	2107	5517	4494
EA	2075	1589	5620	697	1897	1881	5641	2002	1793	2015	5909	4536
HSA	2528	1644	5673	778	2305	1584	5206	1592	2158	1496	4861	3585
HSA	2297	1510	5862	727	2235	1683	4996	1659	2141	1583	4788	3471
HSA	2383	1520	5980	729	2314	1745	4757	1669	2089	1436	4721	3600
HSA	2285	1598	5995	763	2096	1686	5003	1573	1942	1516	4653	3776
HSA	2321	1568	5985	761	2352	1771	5197	1693	2164	1464	4917	3675
BSA	2415	1322	5558	1150	2228	1678	5190	3441	2469	1922	4608	8150
BSA	2086	1439	5472	1194	2226	1702	5513	3184	2447	1904	4754	8083
BSA	1989	1498	5443	1182	2087	1752	5366	3395	2488	1855	4723	8225

BSA	2271	1494	5610	1172	2242	1715	5249	3401	2528	1841	4773	8236
BSA	2016	1438	5520	1220	2292	1818	4914	3355	2485	1863	4703	8051
Myo	2286	1874	5584	385	2037	1757	5739	436	2028	1884	4675	533
Myo	2172	1737	5968	362	2043	1736	5451	421	1950	1815	4880	531
Myo	2303	1757	5941	380	2014	1882	5439	420	2097	1905	4779	504
Myo	2190	1870	5965	387	1938	1875	5489	421	1922	1869	4659	532
Myo	2276	1880	5901	384	1844	1879	5489	419	2000	1896	4733	525
HRP	2593	1638	5737	399	2083	1809	5646	483	2144	1651	5794	652
HRP	2137	1748	5704	378	2094	1784	5907	467	2036	1786	5783	632
HRP	2323	1668	6096	380	2233	1697	5644	484	2123	1807	5431	635
HRP	2348	1738	6009	380	2053	1858	5783	478	2210	1776	5481	643
HRP	2391	1698	5798	384	2076	1643	5680	475	2170	1676	5609	615
Hem	2152	1978	4981	455	1666	2411	3698	1016	896	3228	1901	2324
Hem	1928	2044	4856	461	1683	2271	3653	1038	886	3207	1946	2204
Hem	2059	1902	4773	454	1604	2310	3655	1028	912	3448	1871	2172
Hem	2056	1911	4779	446	1628	2333	3898	996	852	3230	1895	2090
Hem	1969	1958	4954	452	1584	2235	3441	1019	886	3257	1824	2198
TRF	2434	1720	5986	774	2237	2038	5276	2262	2225	2240	4680	8890
TRF	2220	1718	5631	754	2273	2108	5088	2327	2262	2160	4785	9006
TRF	2230	1742	5515	743	2220	2106	5189	2144	2156	2376	4572	8959
TRF	2246	1727	5653	758	2184	2026	5208	2249	2182	2220	4838	8971
TRF	2083	1803	5638	768	2022	2031	5093	2201	2232	2321	4713	8956

Table S8 Proteins secondary structure information of untreated and the plasma treated proteins using self-consistent method.

Proteins	α-helix	β-beta	β-turn	Polypro II helix	Random coil	Total
Cyt c	0.38	0.01	0	0.01	0.6	1
Cyt c_p	0.07	0.46	0	0.01	0.46	1
Lys	0.273	0.154	0.133	0.059	0.37	0.989
Lys_p	0.223	0.169	0.126	0.068	0.373	0.959
Pap	0.081	0.255	0.124	0.076	0.471	1.007
Pap_p	0.34	0.153	0.12	0.054	0.342	1.009
Try	0.057	0.183	0.117	0.1	0.547	1.004
Try_p	0.03	0.156	0.107	0.087	0.611	0.991
Pep	0.03	0.156	0.107	0.087	0.611	0.991
Pep_p	0.03	0.156	0.107	0.087	0.611	0.991
EA	0.204	0.251	0.112	0.055	0.401	1.023
EA_p	0.154	0.217	0.118	0.07	0.429	0.988
HSA	0.45	0.112	0.13	0.03	0.292	1.014
HSA_p	0.359	0.147	0.076	0.016	0.435	1.033
BSA	0.519	0.091	0.108	0.046	0.246	1.01

BSA_p	0.385	0.156	0.124	0.041	0.326	1.032
Myo	0.702	0.04	0.068	0.024	0.188	1.022
Myo_p	0.196	0.194	0.114	0.057	0.371	0.932
HRP	0.29	0.151	0.138	0.053	0.367	0.999
HRP_p	0.223	0.17	0.119	0.062	0.39	0.964
Hem	0.29	0.01	0.18	0	0.52	1
Hem_p	0.09	0.01	0.46	0	0.43	0.99
TRF	0.153	0.25	0.115	0.073	0.361	0.952
TRF_p	0.174	0.157	0.143	0.082	0.421	0.977

The subscript "p" represents the plasma treated proteins.

Table S9 Training matrix of the response patterns against proteins in human serum at 2 different concentration levels based on the response from the quadruple-channel optical sensor.

Protein	50 nM				100 nM			
	Ph	RLS	FL	IF	Ph	RLS	FL	IF
Human serum	4232	3717	4680	5366	4383	3743	4939	4792
Human serum	4168	3778	4712	5400	4385	3437	4945	4837
Human serum	4016	3774	4653	5339	4336	3599	5265	4722
Human serum	4153	3879	4694	5452	4218	3694	5232	4765
Human serum	4110	3826	4652	5339	4185	3618	5014	4867
Cyt	2892	3568	3459	4897	1818	3124	2859	4678
Cyt	2823	3660	3526	4909	1777	3318	2759	4736
Cyt	2819	3614	3500	4833	1816	3378	2755	4664
Cyt	2857	3668	3592	4887	1809	3302	2841	4743
Cyt	2855	3610	3496	4901	1807	3412	2815	4668
HSA	5258	3283	4902	6003	5102	3134	5184	6156
HSA	5277	3393	4852	5962	5050	3142	4986	6067
HSA	5364	3384	4832	6004	4873	3318	5193	6118
HSA	5338	3415	4928	5949	5011	3354	5109	6114
HSA	5385	3448	4945	6001	5198	3350	5006	6062
Myo	4458	3424	4432	4842	4649	3165	4871	4284
Myo	4646	3364	4337	4881	4361	3260	4583	4397
Myo	4603	3475	4342	5042	4362	3389	4563	4236
Myo	4536	3429	4411	4940	4458	3277	4697	4256
Myo	4461	3440	4633	4988	4363	3294	4552	4232
Hem	2705	4171	3046	4439	2052	4408	2671	3746
Hem	2738	4356	3061	4358	2088	4487	2542	3740
Hem	2530	4299	2948	4489	2074	4403	2583	3797
Hem	2705	4354	3122	4428	1997	4591	2628	3753
Hem	2511	4221	2987	4376	2041	4578	2620	3827
TRF	5374	3550	4915	5644	5530	3412	5220	6481

TRF	5474	3582	4794	5550	5334	3668	5205	6385
TRF	5436	3478	4875	5751	5537	3625	5231	6447

Table S10 Training matrix of the response patterns against proteins in urine at 2 different concentration levels based on the response of the quadruple-channel optical sensor.

Protein	50 nM				100 nM			
	Ph	RLS	FL	IF	Ph	RLS	FL	IF
Urine	3006	2111	3998	5495	2376	1856	7225	1306
Urine	3168	2036	3966	5365	2490	1810	7331	1311
Urine	3180	2069	3947	5415	2639	1803	7378	1359
Urine	3034	1992	4011	5425	2427	1790	7409	1334
Urine	3021	1986	4096	5375	2537	1969	7433	1345
Cyt c	2084	2647	3150	4204	1399	3966	3839	1217
Cyt c	2247	2644	3178	4368	1391	4267	3882	1195
Cyt c	2090	2635	3199	4435	1433	4247	3933	1236
Cyt c	2268	2645	2996	4482	1290	4057	3973	1177
Cyt c	2137	2603	3025	4413	1351	4137	4026	1217
Lys	3078	2665	3950	5279	2469	3945	6748	2227
Lys	2861	2630	3822	5299	2483	4091	6715	2193
Lys	3019	2530	4024	5481	2609	3793	6579	2161
Lys	3013	2652	3972	5432	2682	3914	6682	2175
Lys	2916	2646	4065	5395	2564	3916	6701	2115
Try	2819	2226	3888	5305	2465	3248	5887	2507
Try	2949	2300	4116	5465	2303	3586	5938	2533
Try	3022	2379	4114	5525	2285	3306	6040	2537
Try	2964	2257	4013	5330	2385	3524	5954	2615
Try	2810	2284	4082	5397	2500	3327	5969	2600
Pep	2862	1666	3703	5443	2536	2147	6838	1565
Pep	3027	1650	3877	5563	2616	2120	6888	1526
Pep	3051	1652	3953	5551	2585	2038	6723	1481
Pep	2898	1647	3750	5532	2686	2079	7009	1479
Pep	2835	1713	3722	5645	2620	2154	6989	1474
HSA	3308	1736	4062	5887	3612	1892	6540	2211
HSA	3475	1691	3958	5709	3722	1849	6475	2232
HSA	3363	1717	4176	5602	3766	1798	6409	2149
HSA	3361	1760	4173	5781	3545	1870	6706	2282
HSA	3420	1770	4152	5645	3661	1823	6573	2199
Myo	3375	1967	3472	4871	2626	2068	6492	1378
Myo	3119	2019	3590	5066	2821	1940	6304	1409
Myo	3391	1998	3637	5015	2614	1866	6352	1364
Myo	3111	1926	3626	5075	2748	2026	6526	1323

Myo	3336	1925	3604	4818	2785	1918	6280	1326
Hem	2066	2457	1894	1995	1307	2715	2825	1608
Hem	2200	2395	1906	2057	1275	2665	2754	1593
Hem	2149	2256	1802	1975	1223	2788	2698	1554
Hem	2103	2328	1807	1952	1229	2695	2777	1573
Hem	2061	2401	1736	1844	1366	2589	2753	1613
TRF	3272	1903	4250	5692	3070	2418	6758	2212
TRF	3046	1951	4238	5729	3118	2573	6752	2231
TRF	3054	1986	4306	5775	3186	2506	6871	2278
TRF	3320	1895	4452	5790	3145	2413	6682	2160
TRF	3098	2071	4425	5735	3079	2359	6748	2179

Table S11 Training matrix of the response patterns against proteins in cells with and without the plasma treatment based on the response from the quadruple-channel optical sensor.

Cell	Without the plasma treated				With the plasma treated			
	Ph	RLS	FL	IF	Ph	RLS	FL	IF
HUVEC	3711	3340	4498	2672	2929	3509	3932	2439
HUVEC	3734	3513	4257	2609	2887	3413	4046	2458
HUVEC	3782	3372	4383	2594	2818	3315	4112	2556
HUVEC	3687	3570	4422	2535	2834	3321	4075	2483
HUVEC	3601	3479	4270	2793	2815	3349	4045	2461
4T1	3804	3659	4725	3081	2953	3640	4146	3174
4T1	3747	3582	4744	3375	3087	3798	3951	3084
4T1	3775	3475	4895	3256	3050	3723	4133	2985
4T1	3785	3479	4878	3110	2974	3696	4115	2939
4T1	3822	3531	5012	3059	2954	3673	3938	2903
MCF-7	3605	3574	4443	2979	2984	3604	4678	3335
MCF-7	3724	3578	4414	3299	3084	3512	4424	3371
MCF-7	3660	3580	4656	3354	2868	3655	4444	3257
MCF-7	3707	3634	4702	3180	2937	3522	4563	3313
MCF-7	3743	3725	4617	3284	2873	3682	4614	3273
H446	2893	2889	4073	735	2619	3235	4054	731
H446	2864	3181	3885	697	2641	2951	4081	716
H446	2825	3014	3633	712	2544	3076	3805	784
H446	2895	3004	3821	730	2438	2984	3918	749
H446	2861	3053	3828	718	2421	3226	3843	742
SKOV-3	3635	3620	4250	2746	2915	3604	4050	2671
SKOV-3	3577	3509	4028	2762	2865	3487	3802	2663
SKOV-3	3832	3736	4116	2779	2853	3501	4063	2675
SKOV-3	3882	3790	4037	2655	3093	3449	3963	2634
SKOV-3	3727	3640	4046	2657	3051	3654	4023	2575

OCI-LY1	2762	2845	4453	463	2584	2818	4043	480
OCI-LY1	2717	2709	4069	471	2705	2803	4029	508
OCI-LY1	2748	2696	4187	493	2604	2827	3927	485
OCI-LY1	2726	2771	4202	506	2524	2700	4052	497
OCI-LY1	2757	2650	3940	501	2676	2974	3824	478
SU-DHL-6	3403	3203	4075	1689	2812	3412	3846	1947
SU-DHL-6	3308	3190	3870	1638	2721	3382	3779	1804
SU-DHL-6	3478	3446	3915	1691	2713	3515	3748	1833
SU-DHL-6	3399	3345	3907	1752	2722	3403	3877	1848
SU-DHL-6	3365	3018	4028	1670	2733	3445	3900	1875

Table S12 Training matrix of the response patterns against proteins at 50 nM based on the quadruple-channel optical sensor corresponding to Fig. 1b in the text.

Protein	$Ph_{Mn\text{-}ZnS}$	$LS_{Mn\text{-}ZnS}$	$FL_{Mn\text{-}ZnS}$	$FL_{Protein}$	Protein	$Ph_{Mn\text{-}ZnS}$	$LS_{Mn\text{-}ZnS}$	$FL_{Mn\text{-}ZnS}$	$FL_{Protein}$
Cyt c	2537	2019	1728	895	HSA	3583	1513	1967	2967
Cyt c	2594	2061	1726	888	HSA	3609	1548	1952	3062
Cyt c	2428	1915	1713	878	HSA	3617	1627	1815	3065
Cyt c	2590	2109	1739	867	HSA	3708	1438	1830	2997
Cyt c	2532	1977	1769	890	HSA	3599	1571	1820	3001
Lys	2928	2166	2253	1030	BSA	3235	1786	1975	5797
Lys	2912	2293	2183	1109	BSA	3445	1773	1853	5929
Lys	3097	2253	2311	1078	BSA	3429	1728	1965	5797
Lys	3143	2174	2155	1089	BSA	3419	1881	1958	5959
Lys	3123	2291	2113	1073	BSA	3470	1754	1972	5904
Pap	2905	1993	2107	1209	Myo	2824	1790	2025	899
Pap	2962	2158	2111	1228	Myo	2970	1831	1910	917
Pap	3092	2025	2284	1195	Myo	2973	1924	1974	908
Pap	3048	1955	2151	1178	Myo	2792	1755	1896	912
Pap	2996	2187	2009	1237	Myo	2890	1777	1842	921
Try	2941	1989	2160	1361	HRP	3024	2062	2467	988
Try	2966	2077	2066	1399	HRP	3122	1974	2435	978
Try	3038	1871	1999	1384	HRP	3285	1994	2272	985
Try	3088	1926	2125	1386	HRP	3281	1967	2292	995
Try	2986	2049	2044	1351	HRP	3113	1875	2406	1007
Pep	3194	1504	2200	1586	Hem	1926	2147	1445	1686
Pep	3153	1647	2006	1565	Hem	2006	2204	1317	1620
Pep	3163	1617	2122	1596	Hem	1993	2301	1426	1605
Pep	2986	1695	2080	1602	Hem	1978	2390	1434	1666
Pep	3088	1648	2282	1599	Hem	1956	2368	1468	1640
EA	2941	1698	2265	3824	TRF	3325	1738	2060	2575
EA	3155	1738	2103	3778	TRF	3342	1719	2030	2632
EA	3186	1755	2139	3814	TRF	3309	1910	2072	2565
EA	3174	1873	2280	3868	TRF	3243	1896	2108	2530
EA	2995	1812	2194	3743	TRF	3447	1874	2006	2568

References

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