Electronic Supplementary Information (ESI):

Lab-on-Graphene: Graphene Oxide as Triple-Channel Sensing Device for Proteins Discrimination

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Experimental details:

Synthesis of graphene oxide (GO): GO with multiple properties was synthesized by a modified Hummers method. First, 0.75 g $NaNO_3$ was added to 34 ml H_2SO_4. Subsequently, 1 g of graphite flake was added to the solution at 0 °C and then the mixture was oxidized by 5 g of $KMnO_4$ with vigorous stirring. After increasing the temperature to 35 °C, the solution was stirred for 2 h. The mixture was diluted by 50 mL distilled water at 0 °C, and stirred for another 2 h. Then, 4 mL of H_2O_2 was slowly added for oxidization and stirred vigorously until no gas was generated. The mixture was then filtered by a cellulose acetate membrane filter and washed with 1:10 aq. HCl (500 mL) and DI water (500 mL). To completely wash the graphite oxide flakes, the resultant graphite oxide was dissolved in water again and centrifuged several times to reach the pH 7. Then graphite oxide was dried under 60°C and dissolved in water to form 1 mg/ml solution.

Characterization: The photoluminescence (PL) spectra of GO in water were measured by spectrofluorometer (JASCO FP-6500). The excitation wavelength was 450 nm. The absorbance of GO and the mixture in water were measured by UV/VIS spectrophotometer (JASCO V-550). Atomic force microscopic (AFM) images were taken using a SPM-9600 (Shimadzu) atomic force microscope. The detection signals were recorded by a microplate reader (Bio Tek, synergy4, America)

***Sensing**:* Target proteins were mixed with GO solution and shaken mildly for three hours. Then the fluorescence intensities (600 nm) and absorbance (405 nm) of the mixture were recorded to obtain the FL and Turbidity signals. Subsequently, TMB and H_2O_2 were added into the mixture, the absorbance at 652 nm was recorded after ten minutes for Colorimetric signals. Then, the data was processed using LDA in SPSS v16.0.

Table S1. Basic properties and manufacturers of proteins.

Protein	MW (kDa)	pI	Metal	Company	Purity
Hemoglobin (Hem) from bovine blood	64.5	6.8	Yes	Sigma	>95%
Pepsin (Pep) 1:3000	35	1~2.5	No	Genview	>85%
Papain (Pap)	23.0	9.6	No	Genview	>98%
Lysozyme (Lys)	14.4	9.6~11	No	Genview	>95%
Myoglobin (Myo)	17.0	7.2	Yes	Sigma	>95%
Transferrin (TRF) from human blood	~75	5.6	Yes	Sigma	>98%
Human serum albumin (HSA)	69.4	5.2	No	Sigma	>95%
Bovine serum albumin (BSA)	66.3	4.8	No	Sigma	>95%

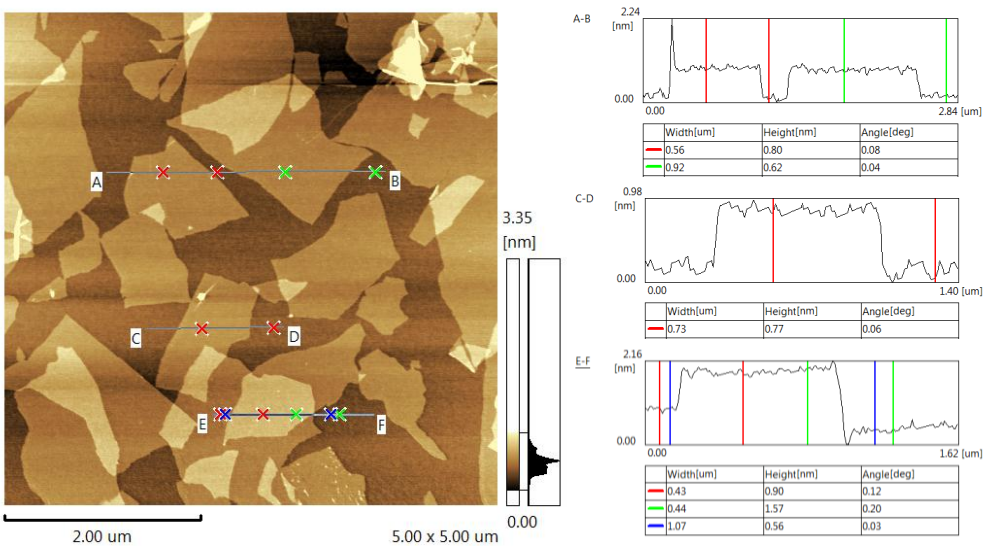

Fig. S1: AFM images of GO sheets. GO sheets are single layer as the height is about 0.8nm and the diameter is about 2μm

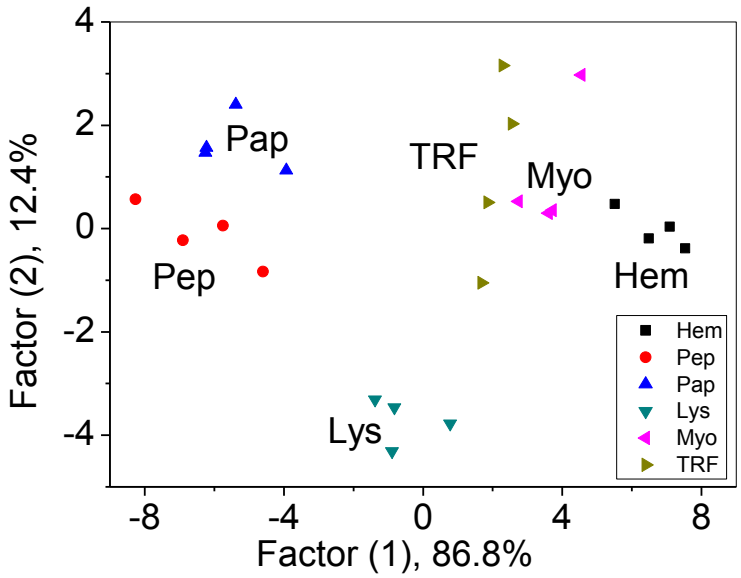

Fig. S2: Canonical score plot for the three-channel patterns as obtained from LDA for six proteins at 500 nM.

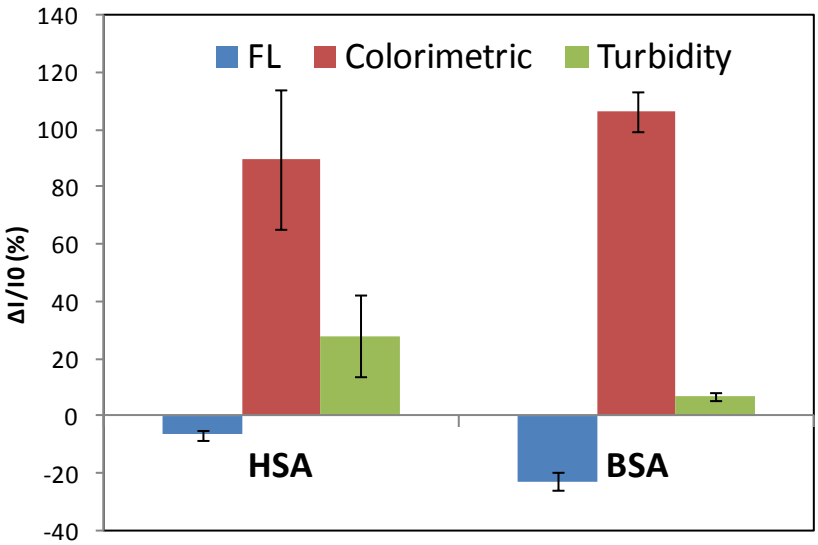

Fig. S3: Fingerprints of HSA and BSA (1μM) based on the patterns of the corresponding values of $\Delta I/I_0$ obtained from the FL, Colorimetric and Turbidity. 8 samples were separated into two respective groups with 100% accuracy.

We further investigated the three proteins system (Hem, Pep and Lys) at two concentrations (500 nM and 1 μM) and found that proteins at different concentrations still could be discriminated (Figure 6B). We found that the accuracy of discriminating different kinds of proteins is still 100%. The discrimination accuracy of 15 unknown samples was found to be 80% (Table S8-9). The only identification fault came from discriminating proteins at different concentrations. That means when the protein concentration is very low, as the difference between proteins at two concentrations is too small to discriminate, the discramination ability of the sensor array is limited. The discramination ability of the sensor array is depend on the linear range of each sensing element and the measurement accuracy. In our experiment, some measurement procedures, such as the sample preparation, adding and mixing, were finished by hands, which might lower the measurement accuracy. If the measurement accuracy is improved, more samples could be discriminated successfully. The results have shown that our sensor could discriminate different kinds of protiens and have the potential to discriminate proteins at different concentrations.

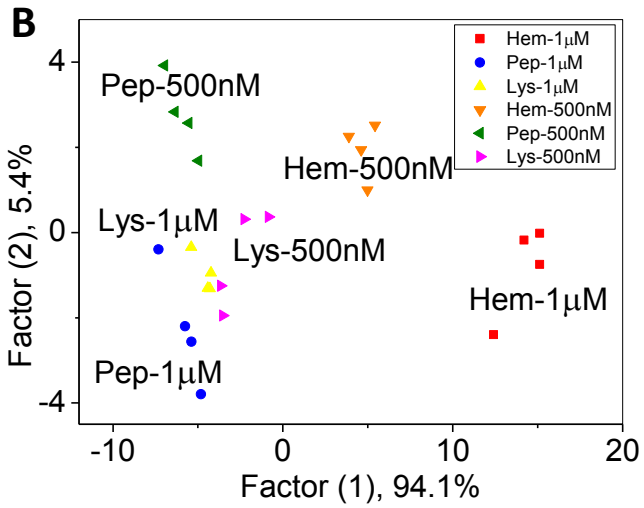

Fig. S4: Canonical score plot for the three-channel patterns as obtained from LDA for Hem, Pep and TRF at different concentrations.

Table S2. Training Matrix of the Response Patterns against Proteins at 1 μM.

Protein	FL	Corlor	Turbidity
Hem	877	1.571	0.783
Hem	982	1.890	1.163
Hem	987	1.821	1.045
Hem	914	1.860	1.044
Pep	1541	0.078	0.800
Pep	1635	0.112	0.794
Pep	1843	0.092	0.798
Pep	1678	0.100	0.823
Pap	1881	0.398	0.760
Pap	1880	0.322	0.807
Pap	2082	0.327	0.823
Pap	1949	0.361	0.800
Lys	1641	0.332	0.630
Lys	1649	0.286	0.748
Lys	1639	0.287	0.678
Lys	1760	0.254	0.785
Myo	1369	0.972	0.785
Myo	1571	0.781	0.804
Myo	1432	0.999	0.881
Myo	1571	0.642	0.872
TRF	1300	0.299	0.658
TRF	1151	0.445	0.739
TRF	1237	0.253	0.792
TRF	1021	0.501	0.797

Table S3. Detection and Identification of Unknown Protein Samples at 1 μM.

	FL	Corlor	Turbidity	Identification	Verification
1	1075	2.444	1.157	Hem	Hem
2	1207	1.519	0.888	Hem	Hem
3	1206	2.120	1.045	Hem	Hem
4	1715	0.080	0.813	Pep	Pep
5	1915	0.088	0.798	Pep	Pep
6	1873	0.082	0.812	Pep	Pep
7	1937	0.396	0.883	Pap	Pap
8	1945	0.409	0.880	Pap	Pap
9	1677	0.256	0.788	Lys	Lys
10	1612	0.395	0.760	Lys	Lys
11	1327	1.130	0.676	Myo	Myo
12	1542	0.598	0.897	Myo	Myo
13	1562	1.004	0.805	Myo	Myo
14	1434	0.923	0.779	Myo	Myo
15	956	0.242	0.723	TRF	TRF

Table S4. Training Matrix of the Response Patterns against Proteins at 500 nM.

Protein	FL	Corlor	Turbidity
Hem	1530	1.162	0.871
Hem	1417	1.167	0.900
Hem	1474	1.205	0.838
Hem	1464	1.314	0.804
Pep	2076	0.395	0.800
Pep	1992	0.373	0.823
Pep	1876	0.406	0.861
Pep	1951	0.417	0.863
Pap	2024	0.556	0.839
Pap	1911	0.566	0.914
Pap	2010	0.583	0.925
Pap	2024	0.540	0.859
Lys	1608	0.290	0.872
Lys	1588	0.679	0.706
Lys	1655	0.554	0.744
Lys	1659	0.322	0.897
Myo	1609	1.164	0.779
Myo	1627	0.886	0.947
Myo	1662	1.407	0.826
Myo	1590	0.955	0.921
TRF	1669	0.851	1.069
TRF	1728	1.032	1.005
TRF	1668	0.899	0.896
TRF	1628	0.807	0.845

Table S5. Detection and Identification of Unknown Protein Samples at 500 nM.

	FL	Corlor	Turbidity	Identification	Verification
1	1495	1.094	0.959	Hem	Hem
2	1472	1.453	0.791	Hem	Hem
3	1919	0.396	0.836	Pep	Pep
4	1911	0.426	0.879	Pep	Pep
5	1910	0.443	0.833	Pep	Pep
6	2006	0.526	0.883	Pap	Pap
7	1905	0.477	0.851	Pep	Pap
8	1964	0.482	0.950	Pap	Pap
9	1470	0.602	0.805	Lys	Lys
10	1488	0.346	0.855	Lys	Lys
11	1662	1.311	0.844	Myo	Myo
12	1650	1.169	0.771	Myo	Myo
13	1715	0.794	0.969	TRF	TRF
14	1744	0.701	1.083	TRF	TRF
15	1648	0.753	0.985	TRF	TRF

Table S6. Training Matrix of the Response Patterns against two Proteins at 500 nM and 1μM.

Protein	FL	Corlor	Turbidity
1 μM Hem	877	1.571	0.783
1 μM Hem	982	1.890	1.163
1 μM Hem	987	1.821	1.045
1 μM Hem	914	1.860	1.044
1 μM Pep	1541	0.078	0.800
1 μM Pep	1635	0.112	0.794
1 μM Pep	1843	0.092	0.798
1 μM Pep	1678	0.100	0.823
500 nM Hem	1530	1.162	0.871
500 nM Hem	1417	1.167	0.900
500 nM Hem	1474	1.205	0.838
500 nM Hem	1464	1.314	0.804
500 nM Pep	2076	0.395	0.800
500 nM Pep	1992	0.373	0.823
500 nM Pep	1876	0.406	0.861
500 nM Pep	1951	0.417	0.863

Table S7. Detection and Identification of Unknown Protein Samples at 500 nM and 1μM.

	FL	Corlor	Turbidity	Identification	Verification
1	1075	2.444	1.157	1 μM Hem	1 μM Hem
2	1207	1.519	0.888	1 μM Hem	1 μM Hem
3	1206	2.120	1.045	1 μM Hem	1 μM Hem
4	1715	0.080	0.813	1 μM Pep	1 μM Pep
5	1915	0.088	0.798	1 μM Pep	1 μM Pep
6	1873	0.082	0.812	1 μM Pep	1 μM Pep
7	1495	1.094	0.959	500 nM Hem	500 nM Hem
8	1472	1.453	0.791	500 nM Hem	500 nM Hem
9	1919	0.396	0.836	500 nM Pep	500 nM Pep
10	1911	0.426	0.879	500 nM Pep	500 nM Pep
11	1910	0.443	0.833	500 nM Pep	500 nM Pep

Table S8. Training Matrix of the Response Patterns against three Proteins at 500 nM and 1μM.

Protein	FL	Corlor	Turbidity
1 μM Hem	877	1.571	0.783
1 μM Hem	982	1.890	1.163
1 μM Hem	987	1.821	1.045
1 μM Hem	914	1.860	1.044
1 μM Pep	1541	0.078	0.800
1 μM Pep	1635	0.112	0.794
1 μM Pep	1843	0.092	0.798
1 μM Pep	1678	0.100	0.823
1 μM Lys	1641	0.332	0.630
1 μM Lys	1649	0.286	0.748
1 μM Lys	1639	0.287	0.678
1 μM Lys	1760	0.254	0.785
500 nM Hem	1530	1.162	0.871
500 nM Hem	1417	1.167	0.900
500 nM Hem	1474	1.205	0.838
500 nM Hem	1464	1.314	0.804
500 nM Pep	2076	0.395	0.800
500 nM Pep	1992	0.373	0.823
500 nM Pep	1876	0.406	0.861
500 nM Pep	1951	0.417	0.863
500 nM Lys	1608	0.290	0.872
500 nM Lys	1588	0.679	0.706
500 nM Lys	1655	0.554	0.744
500 nM Lys	1659	0.322	0.897

Table S9. Detection and Identification of Unknown Protein Samples at 500 nM and 1μM.

	FL	Corlor	Turbidity	Identification	Verification
1	1075	2.444	1.157	1 μM Hem	1 μM Hem
2	1207	1.519	0.888	500 nM Hem	1 μM Hem
3	1206	2.120	1.045	1 μM Hem	1 μM Hem
4	1715	0.080	0.813	1 μM Pep	1 μM Pep
5	1915	0.088	0.798	500 nM Pep	1 μM Pep
6	1873	0.082	0.812	1 μM Pep	1 μM Pep
7	1677	0.256	0.788	1 μM Lys	1 μM Lys
8	1612	0.395	0.760	500 nM Lys	1 μM Lys
9	1495	1.094	0.959	500 nM Hem	500 nM Hem
10	1472	1.453	0.791	500 nM Hem	500 nM Hem
11	1919	0.396	0.836	500 nM Pep	500 nM Pep
12	1911	0.426	0.879	500 nM Pep	500 nM Pep
13	1910	0.443	0.833	500 nM Pep	500 nM Pep
14	1470	0.602	0.805	500 nM Lys	500 nM Lys
15	1488	0.346	0.855	500 nM Lys	500 nM Lys