Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Plasmon Resonance-Inspired Discriminator Unscrambles Lipoprotein Subtypes

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2. Experimental section

2.1. Reagents and chemicals

Cytochrome C (Cyt-c), Lysozyme (LZ), Hemoglobin (Hb), Myoglobin (Mb), Human serum albumin (HSA), Trypsin (Tps), α-Casein, Ovalbumin (Ova), γ-Globulin (γ-Glo) and Immunoglobulin G (IgG) were purchased from Sigma-Aldrich (St. Louis, USA). Low-Density Lipoprotein (LDL), Oxidized Low-Density Lipoprotein (Ox-LDL), High-Density Lipoprotein (HDL), and Very Low-Density Lipoprotein (VLDL) were received from Yiyuan Biotech Co (Guangzhou, China). Chloroauric acid (HAuCl4·4H2O), ascorbic acid (AA), silver nitrate (AgNO3) and HCl were obtained from Sinopharm Chemical Reagent Co., Ltd (Shenyang, China). Hexadecyl trimethyl ammonium bromide (CTAB) was obtained from Dalian Meilun Biotechnology Co., Ltd (Dalian, China). Sodium borohydride (NaBH4) was obtained from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Poly(sodium-p-styrenesulfonate) (PSS) was obtained from Yuanye Bio-Technology Co., Ltd (Shanghai, China). All the reagents were at least of analytical reagent grade and used without further purification. The anti-LDL aptamer fragment with 40 bases (5'-ACCT CGAT TTTA TATT ATTT CGCT TACC AACA ACTG CAGA-3') was determined accroding the reference[1] and synthesized by Sangon Biotechnology Co. Ltd (Shanghai, China). The clinical samples were provided by "The Second Affiliated Hospital of Shenyang Medical College". Deionized (DI) water of 18 MΩ was used throughout. The biological relevant concentrations of LDL, HDL and VLDL in healthy human serum were 1.063-1.03 mg/mL, 1.21-1.063 mg/mL and 1.006 mg/mL, respectively.[2]

2.2. Instrumentation

Transmission electron microscopy (TEM) images were recorded on a Tecnai G20 microscope (Hillsboro, USA). Ultraviolet-visible (UV-vis) absorption spectra were recorded on a UV-3900 Spectrophotometer (Hitachi Corporation). The absorption spectra were recorded on a Synergy H1 microplate reader (Biotek, USA) at room temperature. The zeta potentials were recorded on a Zetasizer Nano S90 (Malvein, UK). The standard clinical test of clinical samples is completed by Roche biochemical analyzer, P800 (Roche, Germany).

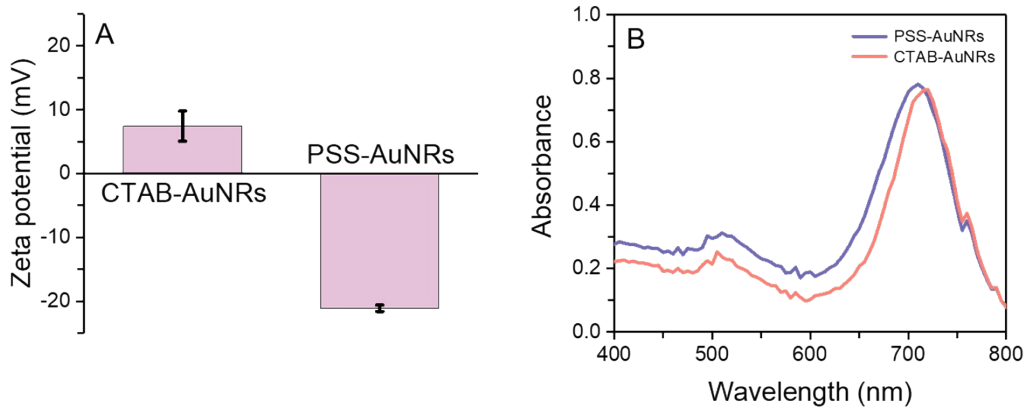

Fig. S1 (A) Zeta potentials of the as-prepared CTAB-AuNRs and PSS-AuNRs dispersed in aqueous solution; (B) UV-Vis absorption spectra of the CTAB-AuNRs and PSS-AuNRs.

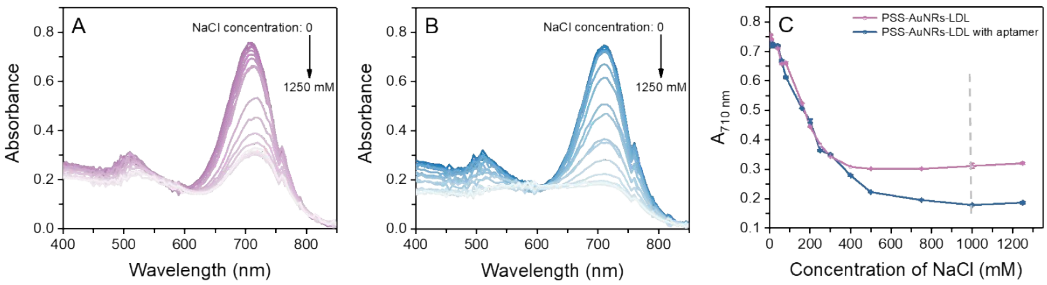

Fig. S2 (A) UV-Vis absorption spectra of PSS-AuNRs dispersions with LDL and various concentrations of NaCl in 5 mM PB buffer (LDL: 7.5 μg/mL); (B) UV-Vis absorption spectra of PSS-AuNRs dispersions with LDL, aptamer and various concentrations of NaCl in 5 mM PB buffer (LDL: 7.5 μg/mL; aptamer: 1 μM); (C) The maximum absorbance values of A710 nm (no aptamer) and A710 nm (with aptamer) for PSS-AuNRs-LDL as a function of various concentrations of NaCl.

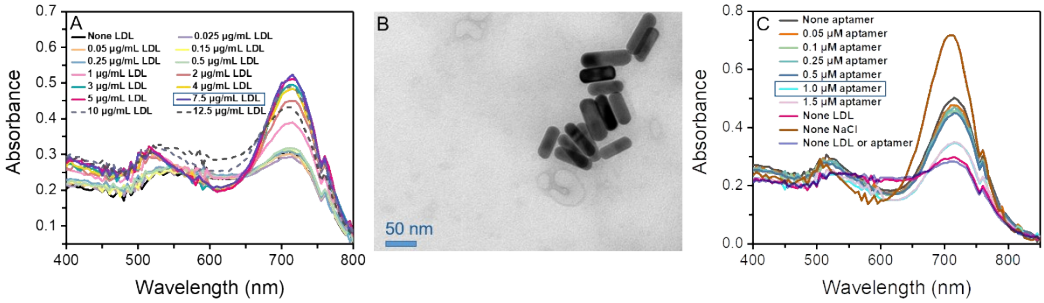

Fig. S3 (A) UV-Vis absorption spectra of PSS-AuNRs dispersions with various concentrations of LDL in PB buffer containing 1000 mM NaCl; (B) The transmission electron microscope (TEM) images of PSS-AuNRs with excessive LDL in 1000 mM NaCl; (C) UV-Vis absorption spectra of PSS-AuNRs-LDL dispersions with various concentrations of aptamer in PB buffer containing 1000 mM NaCl (LDL: 7.5 μg/mL).

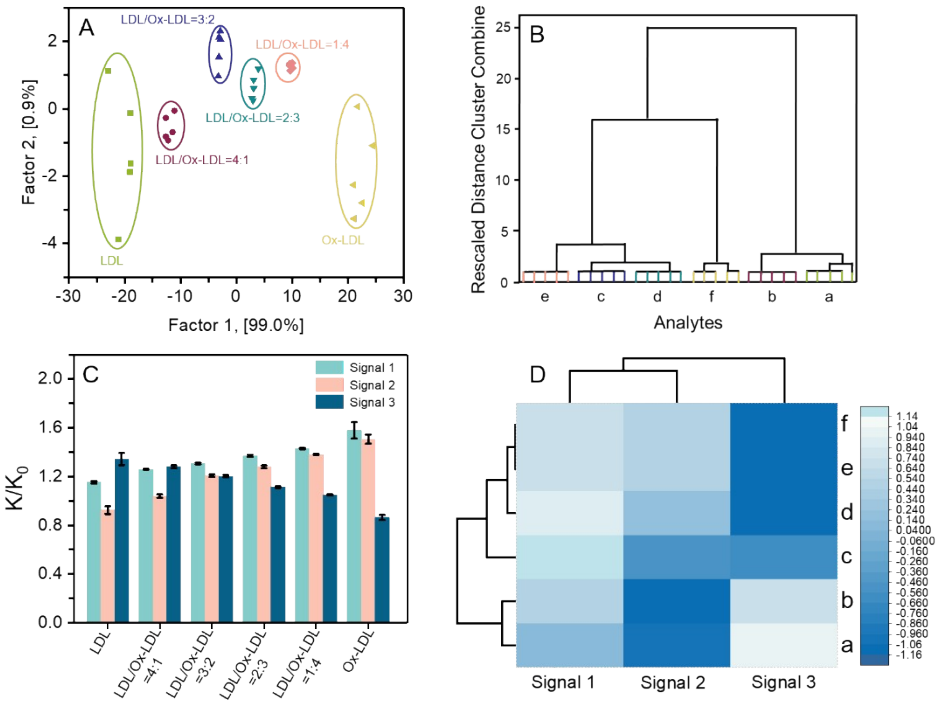

Fig. S4 (A) LDA plot and (B) HCA dendrogram for the mixture of LDL and Ox-LDL, as well as pure LDL and Ox-LDL; (C) The fingerprints of the binary mixture based on the patterns of the corresponding values of K/K_0; (D) The cluster analysis heat map derived from the discriminator platform (a: LDL; b: LDL/Ox-LDL=4:1; c: LDL/Ox-LDL=3:2; d: LDL/Ox-LDL=2:3; e: LDL/Ox-LDL=1:4; f: Ox-LDL).

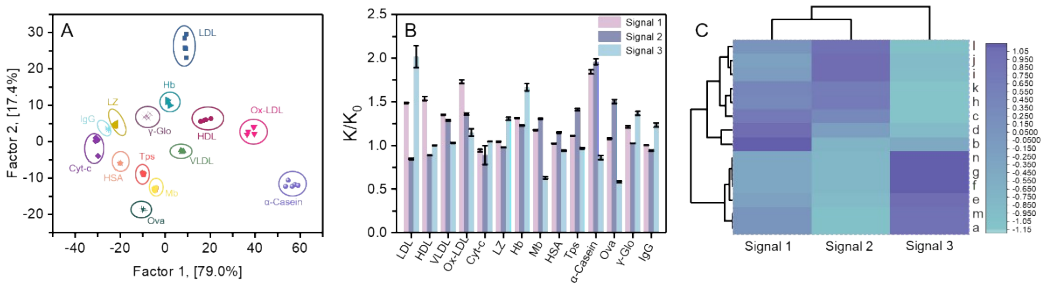

Fig. S5 (A) LDA plot and (B) the fingerprint of LDL, HDL, VLDL, Ox-LDL, Cyt-c, LZ, Hb, Mb, HSA, Tps, α-Casein, Ova, γ-Glo and IgG; (C) The cluster analysis heat map derived from the discriminator platform (a: LDL; b: HDL; c:VLDL; d: Ox-LDL; e: Cyt-c; f: LZ; g: Hb; h: Mb; i: HSA; j: Tps; k: α-Casein; l: Ova; m: γ-Glo; n: IgG).

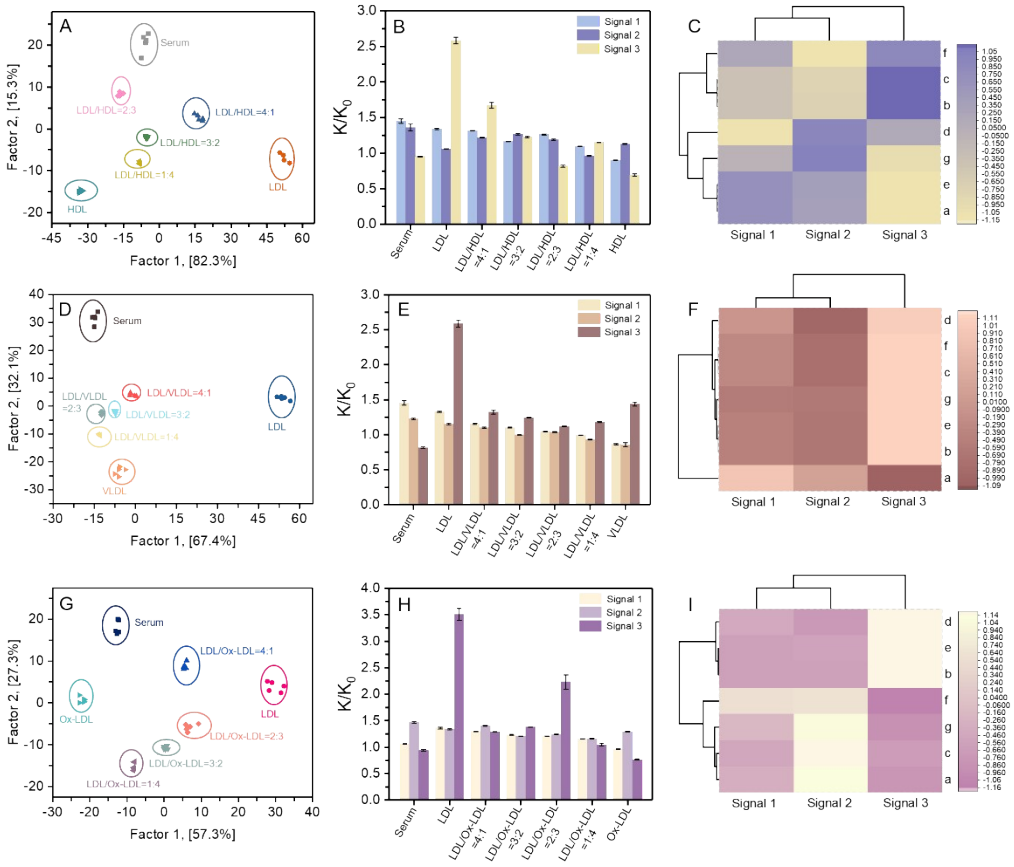

Fig. S6 (A) LDA plot for the mixture of LDL/HDL, pure LDL and HDL in human serum; (B) The fingerprints of the binary mixture LDL/HDL based on the patterns of the corresponding values of K/K_0; (C) The cluster analysis heat map derived from the discriminator (a: serum; b: LDL; c: LDL/HDL=4:1; d: LDL/HDL=3:2; e: LDL/HDL=2:3; f: LDL/HDL=1:4; g: HDL); (D) LDA plot for the mixture of LDL/VLDL, pure LDL and VLDL in human serum; (E) The fingerprints of the binary mixture LDL/VLDL based on the patterns of the corresponding values of K/K_0; (F) The cluster analysis heat map derived from the discriminator (a: serum; b: LDL; c: LDL/VLDL=4:1; d: LDL/VLDL=3:2; e: LDL/VLDL=2:3; f: LDL/VLDL=1:4; g: VLDL); (G) LDA plot for the mixture of LDL/Ox-LDL, pure LDL and Ox-LDL in human serum; (H) The fingerprints of the binary mixture based on the patterns of the corresponding values of K/K_0; (I) The cluster analysis heat map derived from the discriminator (a: serum; b: LDL; c: LDL/Ox-LDL=4:1; d: LDL/Ox-LDL=3:2; e: LDL/Ox-LDL=2:3; f: LDL/Ox-LDL=1:4; g: Ox-LDL).

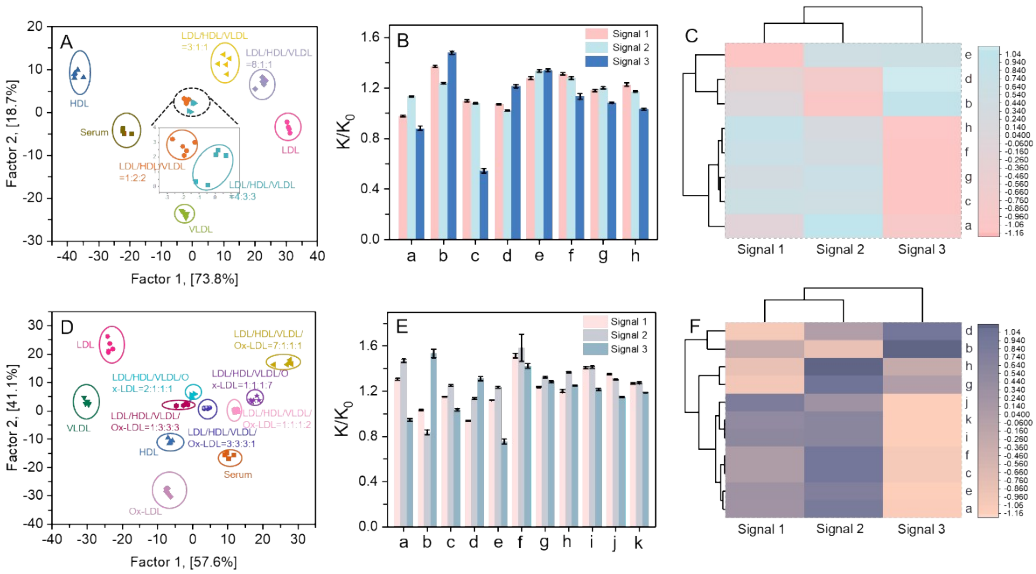

Fig. S7 (A) LDA plot for the mixture of LDL/HDL/VLDL, pure LDL, HDL and VLDL in human serum; (B) The fingerprints of the ternary mixture based on the patterns of the corresponding values of K/K_0; (C) The cluster analysis heat map derived from the discriminator (a: serum; b: LDL; c: HDL; d: VLDL; e: LDL/HDL/VLDL=8:1:1; f: LDL/HDL/VLDL=3:1:1; g: LDL/HDL/VLDL=4:3:3; h: LDL/HDL/VLDL=1:2:2); (D) LDA plot for the mixture of LDL/HDL/VLDL/Ox-LDL, pure LDL, HDL, VLDL and Ox-LDL in human serum; (E) The fingerprints of the quaternary mixture based on the patterns of the corresponding values of K/K_0; (F) The cluster analysis heat map derived from the discriminator (a: serum; b: LDL; c: HDL; d: VLDL; e: Ox-LDL; f: LDL/HDL/VLDL/Ox-LDL=7:1:1:1; g: LDL/HDL/VLDL/Ox-LDL=2:1:1:1; h: LDL/HDL/VLDL/Ox-LDL=1:3:3:3; i: LDL/HDL/VLDL/Ox-LDL=1:1:1:7; j: LDL/HDL/VLDL/Ox-LDL=1:1:1:2; k: LDL/HDL/VLDL/Ox-LDL=3:3:3:1).

Table S1. Physical properties of the proteins adopted in the present study.

Protein	pI	Mw (kDa)
Low density lipoprotein (LDL)	5.5	3000
High density lipoprotein (HDL)	/	300
Very low density lipoprotein (VLDL)	4.3	30000
Oxidized Lipoprotein (Ox-LDL)	/	ca. 2000
Cytochrome C (Cyt-c)	10.8	11
Lysozyme (LZ)	9.6-11.0	14
Hemoglobin (Hb)	6.8	68
Myoglobin (Mb)	6.99	16.7
Human serum albumin (HSA)	4.7-4.9	66
Trypsin (Tps)	10.5	24
α-Casein	4.0	57
Ovalbumin (Ova)	4.5	44.5
γ-Globulin (γ-Glo)	5.8-8.2	200
Immunoglobulin G (IgG)	8.0	150

Table S2. The test results for the clinical serum samples obtained from "The Second Affiliated Hospital of Shenyang Medical College" by spectrophotometry using an Automatic Chemistry Analyzer.

Sample	LDL (mmol/L)	HDL (mmol/L)	Molar ratio of LDL/HDL
1	1.89	1.46	1.29
2	2.51	1.65	1.52
3	2.69	1.63	1.65
4	4.29	1.16	3.70
5	4.46	1.18	3.78
6	4.29	1.13	3.80

References

1. D. Klapak, S. Broadfoot, G. Penner, A. Singh and E. Inapuri, PLoS One, 2018, **13,** e0205460.
2. C. A. Lutomski, S. M. Gordon, A. T. Remaley and M. F. Jarrold, Anal. Chem., 2018, **90,** 6353-6356.