Supporting Information

Competitive Adsorption on Gold Nanoparticle for Human Papillomavirus 16 L1 Protein Detection by LDI-MS

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Preparation of reagents

A stock solution of HPV16 L1 aptamer (20 μM) was prepared in DEPC water. Further dilutions of this solution were prepared in disodium hydrogen phosphate-citrate buffer (pH 3, pH 5, pH 7). A stock solution of 1 M NaCl was prepared by dissolving 40mg in 1ml of DEPC water and, accordingly, was diluted for optimization studies. A standard solution of HPV16 L1 was prepared by dissolving 50 μg of recombinant HPV16 L1 (abcom, USA) in 50 μL of DEPC water. Then dilute with binding buffer to the required concentration.

5-nm Au nanoparticles (AuNPs) were synthesized according to previous report [1] .Briefly, 10 mL of 1 mM HAuCl4 was mixed with 1 mL of 38.8 mM trisodium citrate and vigorously stirred for 15 min. The mixture of 0.4 μg NaBH4 and 0.4 mL trisodium citrate (38.8 mM) was slowly added to the precursor solution and stirred for 2 h.

40-nm Au nanoparticles (AuNPs) were synthesized according to previous report[2]. Briefly, 50 mL of 0.014% HAuCl4·4H2O was boiling, and then 8 mL of 1% trisodium citrate was added to the boiling HAuCl4. The solution was kept boiling for 15 min and then cooled to room temperature under stirring.

The molar concentration of AuNPs was calculated by measuring UV-vis absorbance (Fig S3) using the formula: $C = A_{450}/\varepsilon_{450}$， where ε_{450} is

the molar extinction coefficient at 450 nm. The calculated particle concentration of AuNPs was approximately 2.5 nM.

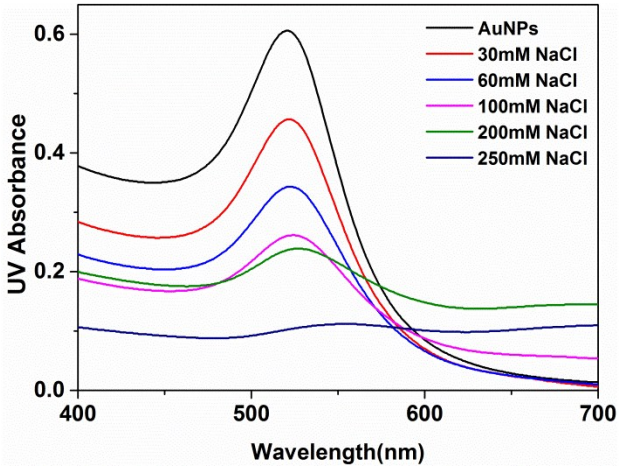

Fig.S1 UV spectrum of AuNPs with various NaCl concentrations

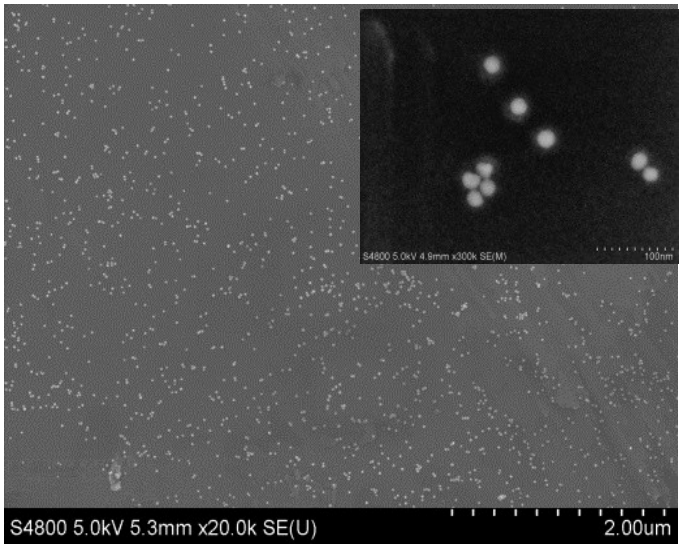

Fig.S2 SEM image of AuNPs(20 nm)

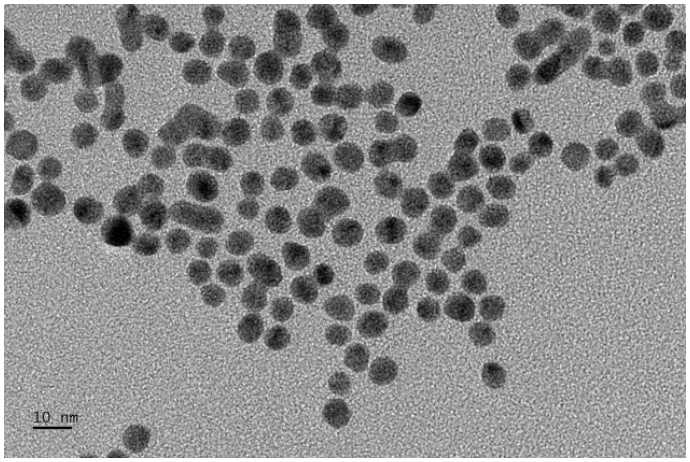

Fig.S3 TEM image of AuNPs(5 nm)

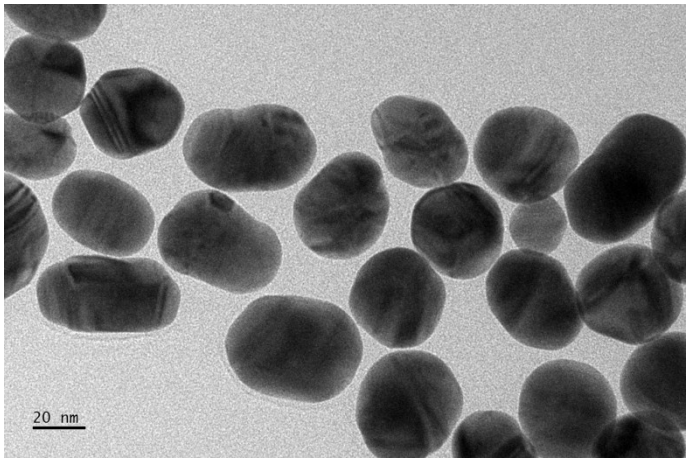

Fig.S4 TEM image of AuNPs(40 nm)

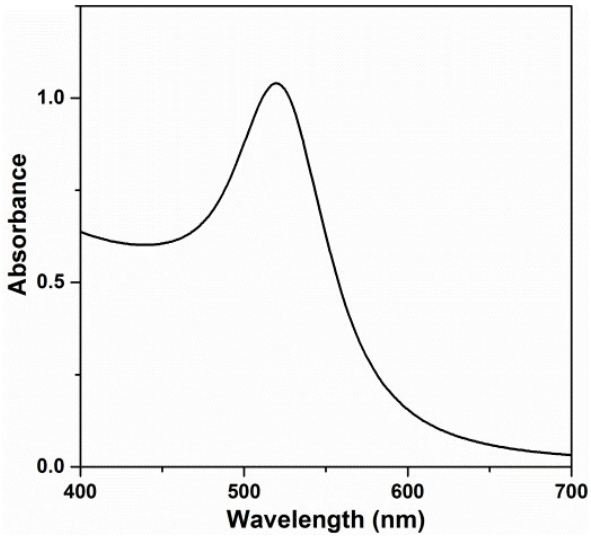

Fig.S5 UV spectrum of AuNPs

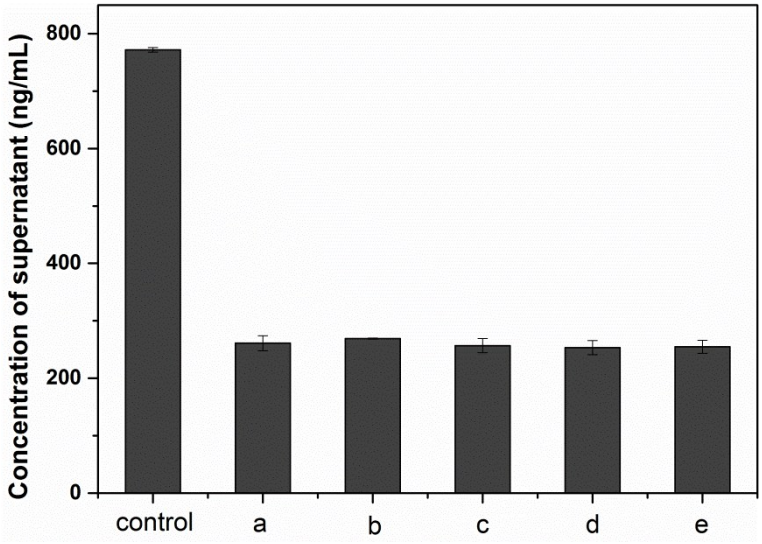

Fig.S6 Concentration of APT $_{HPV\ 16L1}$ (control) and the unattached APT $_{HPV\ 16L1}$after mixing APT $_{HPV\ 16L1}$ with AuNPs (a), with the addition of 0.1 mM K+, Mg2+, tyrosine and cysteine (b-e),

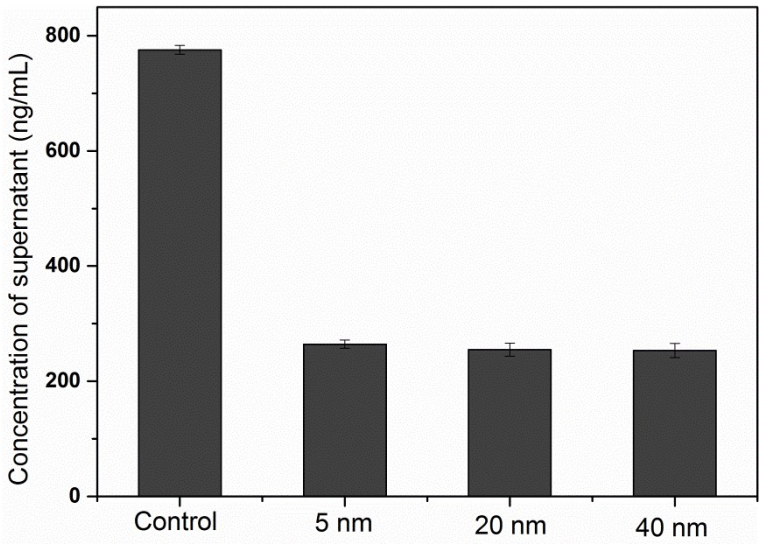

Fig.S7 Concentration of the unattached APT$_{HPV\ 16\ L1}$ after mixing ssDNA with different size of AuNPs (5, 20 and 40 nm).

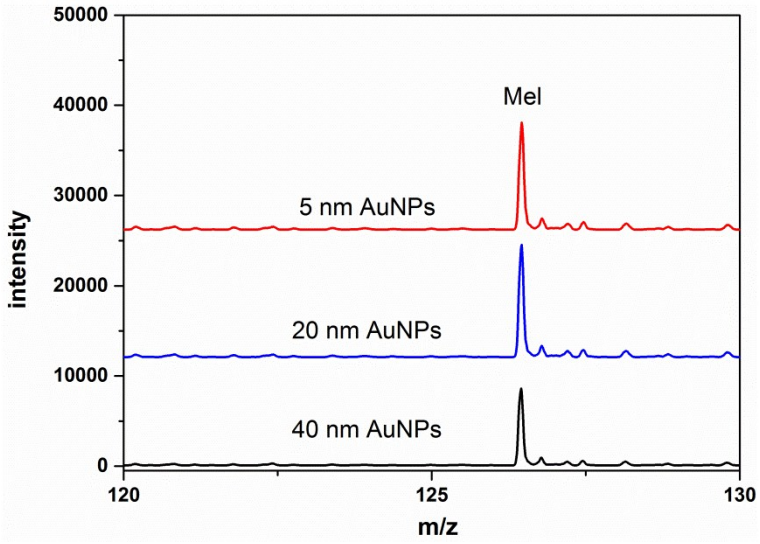

Fig.S8 MS spectrum of Mel in the experiments of aptamer mixing with different size of AuNPs (5, 20 and 40 nm) to detect HPV 16 L1 protein.

Table S1. MS approach of HPV16 L1 calibration solutions.

HPV16 L1 protein Concentration (ng/mL)	Mean ratio for Mel/Mel-CH_3 in three assays	SD
0	0.011	0.003
2	0.551	0.155
10	0.943	0.175
20	1.787	0.280
40	3.384	0.506
60	5.039	0.550
80	6.505	0.506

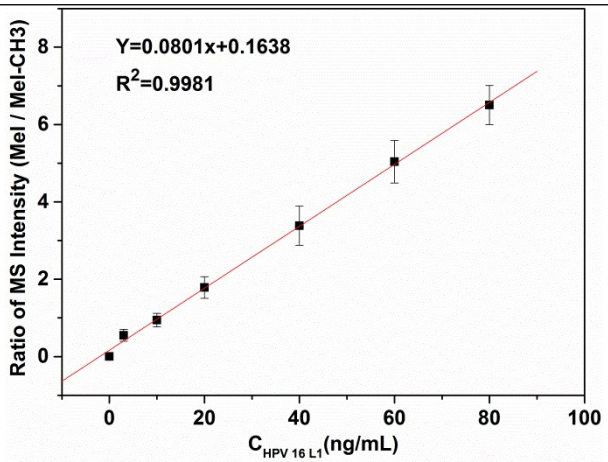

Fig. S9 Linear calibration curve of clinical samples detected by LDI-TOF MS

Table S2 LDI MS approach results for real sample

Sample name	HPV16 L1 protein Concentration (ng/mL)(n=3)	SD
Positive clinical sample	119.8	0.019
Negative clinical sample	n.d.	/
Vaccine sample	33842.0	0.032

n.d. = not detected

Table S3 ELISA of HPV16 L1 calibration solutions

HPV16 L1 protein Concentration (ng/mL)	0	15	30	60	120	240
optical density(OD)	0.019	0.156	0.222	0.339	0.778	1.605
	0.024	0.145	0.213	0.342	0.757	1.602
	0.025	0.137	0.209	0.326	0.746	1.589
	0.021	0.147	0.227	0.352	0.749	1.595

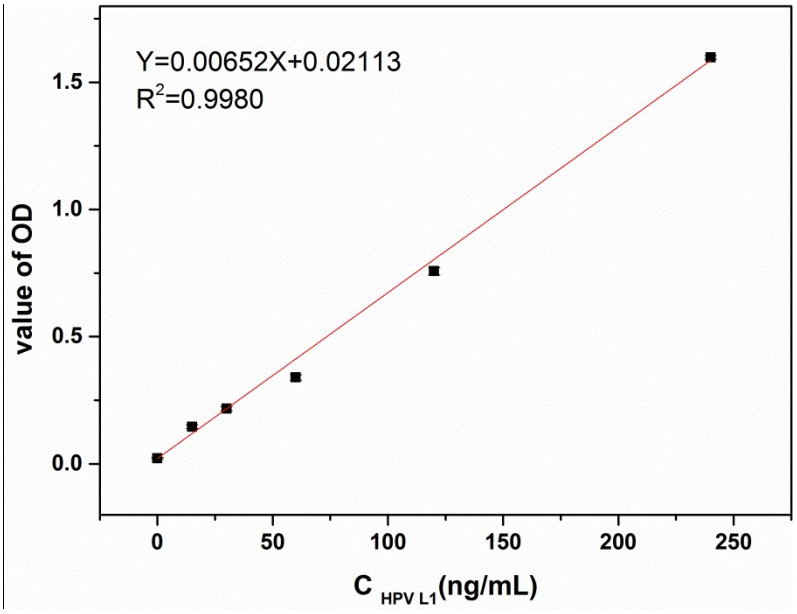

Fig. S10 Linear calibration curve of clinical samples detected by ELISA

Table S4 ELISA approach results for real sample

Sample name	HPV16 L1 protein Concentration (ng/mL) (n=5)	SD
Positive clinical sample	102.5	0.041
Negative clinical sample	n.d.	/
Vaccine sample	37861.8	0.060

n.d.= not detected

Table S5 LDI TOF recoveries for real sample

Sample No.	Sample content (n=3), ng/mL	Added,ng/mL	Found(n=3),ng/mL	RSD(n=3)%	Recoveries%
1	24	5	26	2.8	89.7
2	24	20	48	1.5	109.1
3	24	50	71	1.9	96.0

Reference

[1]Khoa N T, Kim S W, Yoo D H, et al. Size-dependent work function and catalytic performance of gold nanoparticles decorated graphene oxide sheets[J]. Applied Catalysis A General, 2014, 469(3):159–164.

[2] Duan J, Yang M, Lai Y, et al. A colorimetric and surface-enhanced Raman scattering dual-signal sensor for Hg^{2+} based on Bismuthiol II-capped gold nanoparticles[J]. Analytica Chimica Acta, 2012, 723(723):88-93.