

## Supporting Information for:

### A Novel Mass Spectrometry Method Based on Competitive Non-covalent Interaction for the Detection of Biomarkers

Jing Han,<sup>a,b</sup> Yafeng Li,<sup>a</sup> Lingpeng Zhan,<sup>a</sup> Jinjuan Xue,<sup>a</sup> Jie Sun,<sup>a</sup> Caiqiao Xiong <sup>\*a</sup> and  
Zongxiu Nie <sup>\*a,b,c</sup>

<sup>a</sup> Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. E-mail: znie@iccas.ac.cn

<sup>b</sup> University of the Chinese Academy of Sciences, Beijing 100049, China.

<sup>c</sup> National Center for Mass Spectrometry in Beijing, Beijing 100190, China

## Table of contents

1. Experimental.....	S3
2. Fig. S1.....	S7
3. Fig. S2.....	S7
4. Fig. S3.....	S7
5. Fig. S4.....	S8
6. Fig. S5.....	S8
7. Fig. S6.....	S8
8. Fig. S7.....	S9
9. Fig. S8.....	S9
10. Fig. S9.....	S9
11. Fig. S10.....	S10
12. Fig. S11.....	S10
13. Fig. S12.....	S10
14. Fig. S13.....	S11
15. Fig. S14.....	S11
16. Fig. S15.....	S11
17. Fig. S16.....	S12
18. Fig. S17.....	S12
19. Fig. S18.....	S12
20. Table S1.....	S13
21. Table S2.....	S13
22. Table S3.....	S13
23. Table S4.....	S13
24. Table S5.....	S14
25. Table S6.....	S14
26. References.....	S14

## Experimental section

### Reagents and apparatus:

Prostate specific antigen (PSA) from human semen was obtained from Sigma-Aldrich. Tetrachloroauric(III) acid trihydrate, sodium citrate, adenine, guanine, thymine, cytosine and 3-methyl-3H-purin-6-amine were obtained from Beijing InnoChem Science & Technology Co., Ltd. ssDNA (aptamer of PSA) with the following sequence: CGT CGT ATT AAA GCT CGC CAT CAA ATA GCT TT and phosphate buffered saline (PBS) buffer (10 mM, 1×) were obtained from Sangon Biotech (Shanghai) Co., Ltd. Human serum albumin (HSA), immunoglobulin G (IgG), transferrin (TF), and fetal bovine serum (FBS) were provided by Beijing Solarbio Science & Technology Co., Ltd. OliGreen ssDNA quantitation kit was purchased from Beijing Fanbo Biochemicals Co., Ltd. Enzyme linked immunosorbent assay (ELISA) kit for human total PSA was from Abcam Trading (Shanghai) Co., Ltd. Ultrapure water (over 18 MΩ·cm) from a Milli-Q reference system (Millipore) was used throughout.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) was performed on a Bruker Microflex time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a 355 nm and 2 kHz solid state Nd:YAG Smart Beam laser. The mass spectrum was summed up by 200 shots at a laser repetition rate of 1000 Hz and analyzed by flexAnalysis (Bruker Daltonics, Germany). The mass spectra were typically recorded at an accelerating voltage of 19 kV and a reflection voltage of 20 kV with the laser pulse energy of 40 μJ. The fluorescence spectra were measured on microplate reader (Molecular Devices SpectraMax i3). Absorption spectra were made in 1 cm quartz cells with a TU-1900 spectrophotometer (Beijing, China). Transmission electron microscopy (TEM) was recorded on a Hitachi HT7700 transmission electron microscope at 100 kV. X-ray photoelectron spectroscopy (XPS) analysis was conducted by an ESCALAB 250XI X-ray photoelectron spectrometer (Thermo, USA).

### Synthesis and characterization of gold nanoparticle

13-nm Au nanoparticles (AuNPs) were synthesized according to previous reports.<sup>[1,2]</sup> Briefly, 50 mL of 1 mM HAuCl<sub>4</sub> was boiling with vigorous stirring, then 5 mL of 38.8 mM sodium citrate was rapidly added to the above solution and kept boiling for another 10 min to get a wine-red solution, then stopped heating and cooled to room temperature. The AuNPs filtered through a 0.45-μm membrane filter was characterized by UV/Vis spectrometry and transmission electron microscopy (TEM).

5-nm Au nanoparticles (AuNPs) were synthesized according to previous report.<sup>[3]</sup> Briefly, 10 mL of 1 mM HAuCl<sub>4</sub> was mixed with 1 mL of 38.8 mM trisodium citrate and vigorously stirred for 15 min. The mixture of 0.4 μg NaBH<sub>4</sub> and 0.4 mL trisodium citrate (38.8 mM) was slowly added to the precursor solution and stirred for 2 h.

30-nm Au nanoparticles (AuNPs) were synthesized according to previous report.<sup>[4]</sup> Briefly, 50 mL of 0.014% HAuCl<sub>4</sub>·4H<sub>2</sub>O was boiling, and then 8 mL of 1% trisodium citrate was added to the boiling HAuCl<sub>4</sub>. The solution was kept boiling for 15 min and then cooled to room temperature under stirring.

### Optimization of ssDNA binding to gold nanoparticles

To optimize the reaction conditions of pH and salt concentration, AuNPs solutions (1.3 nM, 200 μL) mixed with 1 μM 50 μL ssDNA in sodium phosphate (pH 3.0, 5.0

and 7.4, 10 mM) or ultrapure water were co-incubated overnight. After two centrifugations (12000 rpm) and washing cycles, the concentration of the free ssDNA was determined by adding OliGreen (ssDNA labeling reagent, Molecular Probe,) and then measured the fluorescence with  $\lambda_{\text{ex/em}} = 480/520$  nm.

To optimize the size of AuNPs, different size of AuNPs (5, 13 and 30 nm) was incubated with ssDNA (final concentration 0.1  $\mu\text{M}$ ) overnight. Following the above steps, the concentration of the free ssDNA was measured to test the ability of loading ssDNA on different size of AuNPs.

To optimize the ratio of ssDNA and AuNPs, AuNPs solutions (1.3 nM, 200  $\mu\text{L}$ ) mixed with 1  $\mu\text{M}$  ssDNA (10, 50, 100 and 150  $\mu\text{L}$ ) in pH 3.0 sodium phosphate were co-incubated overnight. Following the above steps, the loading quantity and efficiency were measured.

To test the influence of positively charged species and  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , tyrosine and cysteine with concentration of 0.1 mM were added into the  $\text{Apt}_{\text{PSA}}/\text{AuNPs}$  solution. After incubating overnight, the influence of positively charged species was tested by measuring the unattached ssDNA in the supernatant.

### **Compare the binding affinity of ssDNA-AuNPs and ssDNA-target**

AuNPs solutions (1.3 nM, 200  $\mu\text{L}$ ) were mixed with ssDNA (1  $\mu\text{M}$ , 50  $\mu\text{L}$ ) in sodium phosphate (pH 3.0, 10 mM) and incubated overnight. The mixtures were centrifuged and washed twice, and then the solution was adjusted to 200  $\mu\text{L}$  and separated into two parts. One part was dried in a vacuum. Prostate specific antigen with the final concentration of 40 ng/mL was added into the other part and incubated at room temperature for 1 h. With two centrifugation and washing cycles, the mixtures were also dried in a vacuum. AuNPs solutions in sodium phosphate (pH 3.0, 10 mM) were centrifuged and washed twice, then dried in a vacuum. The powders of AuNPs, AuNPs-ssDNA and AuNPs-ssDNA interacted with PSA, respectively were characterized using X-ray photoelectron spectroscopy (XPS). The intensity of the P 2p peak is dependent on surface coverage of ssDNA on AuNPs. The solutions of AuNPs, AuNPs-ssDNA and AuNPs-ssDNA interacted with PSA, respectively were recorded by absorption spectra. The red shift indicated the level of aggregation of AuNPs.

Albumin and tamm-horsfall protein (1 mg/mL) were added into the  $\text{Apt}_{\text{PSA}}/\text{AuNPs}$  solution and incubated at room temperature for 1 hour. With two centrifugation and washing cycles, the mixtures were separated into two parts. One part was dried in a vacuum and characterized via XPS. The other part was recorded by absorption spectra.

### **Competitive binding of tag and ssDNA to gold nanoparticles**

To an AuNPs suspension (200  $\mu\text{L}$ , 1.3 nM), aptamers (50  $\mu\text{L}$ , 1  $\mu\text{M}$ ) and 10  $\mu\text{L}$ , 1  $\mu\text{M}$  each tag (adenine, guanine, thymine, cytosine) were added and incubated overnight. The solution was centrifuged for 10 min at 12000 rpm with being washed by ultrapure water, then prostate specific antigen with the final concentration of 3 ng/mL was added and incubated at room temperature for 1 h. The solution was centrifuged for 10 min at 12000 rpm and washed three times with ultrapure water. The precipitate was resuspended to 20  $\mu\text{L}$  water.

Control experiment: all steps was the same as above operation, but without added PSA.

In MALDI-TOF MS analysis, 1  $\mu\text{L}$  of the above suspension was premixed with 1  $\mu\text{L}$  matrix (CCA) in a centrifuge tube, and then 1  $\mu\text{L}$  of the resulting mixture was pipetted on the MALDI target plate and air-dried for further MS analysis.

### **The detection of PSA antigen using MALDI-TOF MS**

To an AuNPs suspension (200  $\mu\text{L}$ , 1.3 nM), PSA aptamers (50  $\mu\text{L}$ , 1  $\mu\text{M}$ ) and adenine tag (10  $\mu\text{L}$ , 1  $\mu\text{M}$ ) were added and incubated overnight. The solution was centrifuged for 10 min at 12000 rpm with washes using ultrapure water, then prostate specific antigen with the final concentration of 0.06, 0.3, 0.6, 3 and 6 ng/mL was added and incubated at room temperature for 1 h. The solution was centrifuged for 10 min at 12000 rpm and washed three times with ultrapure water. The precipitate was resuspended to 20  $\mu\text{L}$  water. In MALDI-TOF MS analysis, 1  $\mu\text{L}$  of the above suspension was premixed with 1  $\mu\text{L}$ , 3-Methyladenine (IS, 0.1  $\mu\text{M}$ ) in a centrifuge tube, and then 1  $\mu\text{L}$  of the resulting mixture was added into 1  $\mu\text{L}$  matrix (CCA). Finally, 1  $\mu\text{L}$  of the above mixture was pipetted on the MALDI target plate and air-dried for further MS analysis.

The PSA in healthy urine was removed by a 3 KD Ultra Centrifugal Filter (Merck Millipore, Germany), and the PSA standard was added into the above urine and the dilute urine with the concentration of 20 ng/mL. To aptamer-AuNPs solution, PSA in above two urines were added and incubated for 1 h, then subjected to the proposed method followed by MALDI-MS determination.

### **The specificity testing**

To an AuNPs suspension (200  $\mu\text{L}$ , 1.3 nM), PSA aptamers (50  $\mu\text{L}$ , 1  $\mu\text{M}$ ) and adenine tag (10  $\mu\text{L}$ , 1  $\mu\text{M}$ ) were added and incubated overnight. The solution was centrifuged for 10 min at 12000 rpm with washes using ultrapure water, then human serum albumin (HSA), immunoglobulin G (IgG), transferrin (TF), fetal bovine serum (FBA) with final concentration of 6 ng/mL and the mixture of PSA (0.6 ng/mL final concentration) with FBA was added and incubated at room temperature for 1 h respectively. The solution was centrifuged for 10 min at 12000 rpm and washed three times with ultrapure water. The precipitate was resuspended to 20  $\mu\text{L}$  water. In MALDI-TOF MS analysis, 1  $\mu\text{L}$  of the above suspension mixed with matrix was deposited onto the sampling plate and allowed to dry in air.

Tamm-horsfall protein (T-H protein) as the most abundant protein in urine was also selected to test the specificity of our approach. The mixture of T-H protein (the final concentration of 1 and 10 mg/mL) and PSA (3 ng/mL) were incubated with Apt<sub>PSA</sub>/AuNPs at room temperature for 1 h, respectively. PSA was mixed with Apt<sub>PSA</sub>/AuNPs as control experiment. then subjected to the proposed method followed by MALDI-MS determination.

### **Urine sample analysis**

Fresh human urine of healthy people and patient was obtained from the Peking University People's Hospital (Beijing, China) according to the rules of the local ethical committee. Recovery experiments were conducted by spiking low, middle and high

concentrations (0.8, 8, 50 ng/mL) of PSA targets into healthy people's urine and then subjected to the proposed method followed by MALDI-MS determination. Clinical sample assays were performed by incubating one urine sample of healthy person and four urine samples from patients with aptamer-AuNPs mixture with adenine tags, and then followed the above approach.

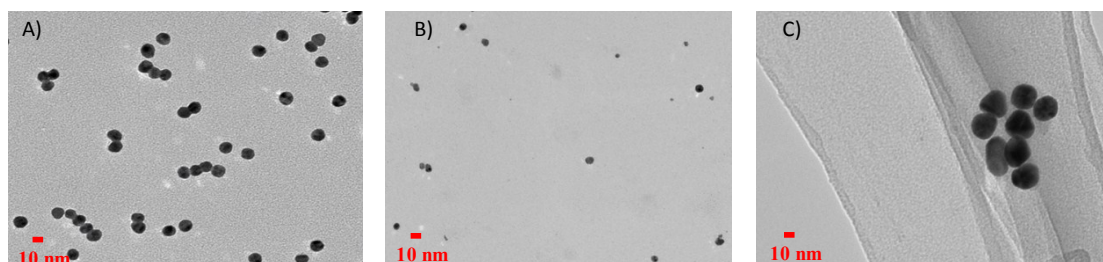
#### **Serum sample analysis**

Serum tests were conducted by spiking various concentrations (3, 6, 15, 30 and 60 ng/mL) of PSA targets into healthy people's serum and then subjected to the proposed method followed by MALDI-MS determination.

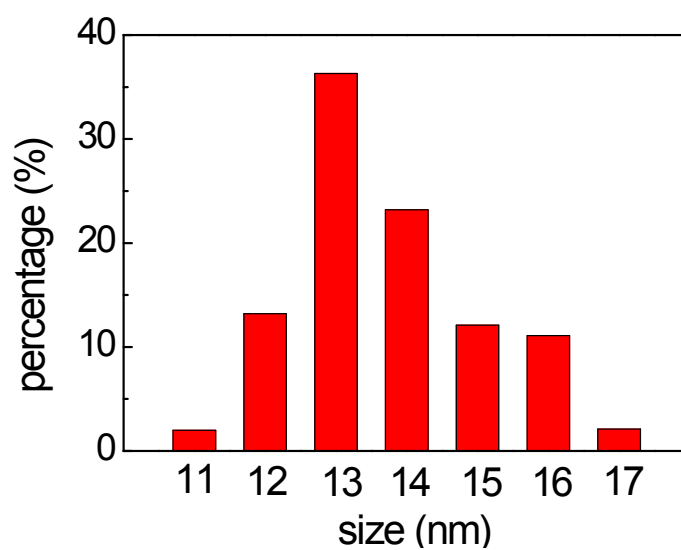
#### **The colorimetric assay**

AuNPs solutions (1.3 nM, 200  $\mu$ L) were mixed with ssDNA (0.1  $\mu$ M, 100  $\mu$ L) and PSA (8 ng/mL) solution with the final concentration of 0.06, 0.3, 0.6, 3 and 6 ng/mL for 30 min. Then NaCl solution (10  $\mu$ L, 30 mM) was added to the mixtures, followed by AuNPs absorption monitoring using the UV/Vis spectrometry.

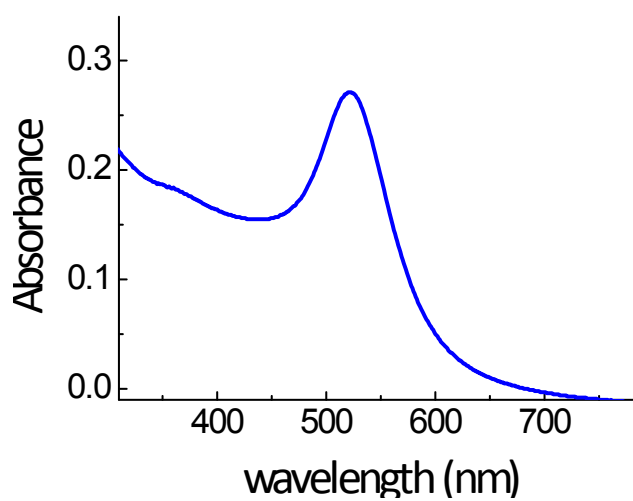
## Supplementary Figures:



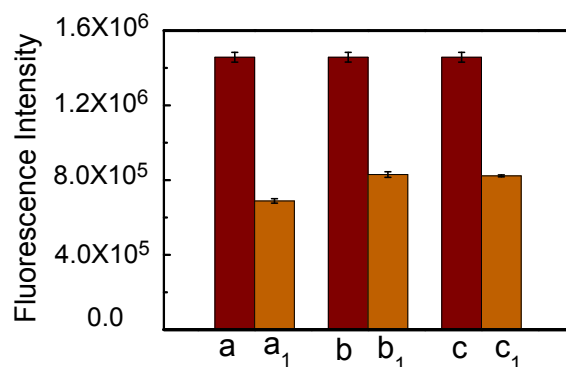
**Fig. S1.** Transmission electron micrograph of citrate-coated AuNPs: (A) 13 nm; (B) 5 nm; (C) 30 nm. Scale bar, 10 nm.



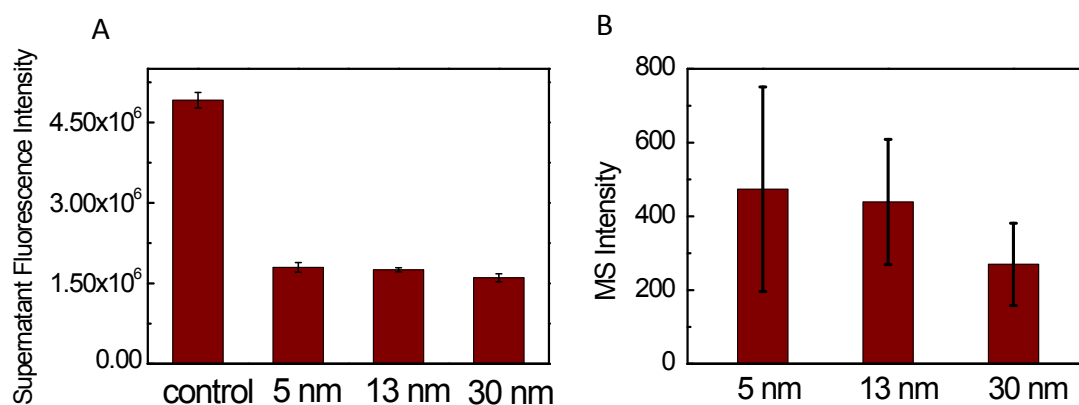
**Fig. S2.** Size distribution of citrate-coated AuNPs (13 nm).



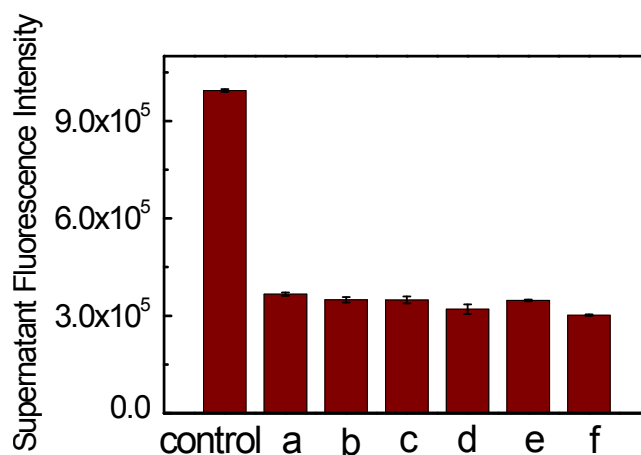
**Fig. S3.** Absorption spectra of citrate-coated AuNPs (13 nm).



**Fig. S4.** Fluorescence intensity of the ssDNA solution (0.1  $\mu$ M) without adding AuNPs (a-c) and the unattached ssDNA after mixing ssDNA (0.1  $\mu$ M) with AuNPs (0.52 nM) (a<sub>1</sub>-c<sub>1</sub>) at different pH: pH 3.0, pH 5.0 and pH 7.4, respectively. The mixtures of ssDNA and AuNPs were centrifuged at speed of 12000 rpm for 10 min to collect the unattached ssDNA from the supernatant.

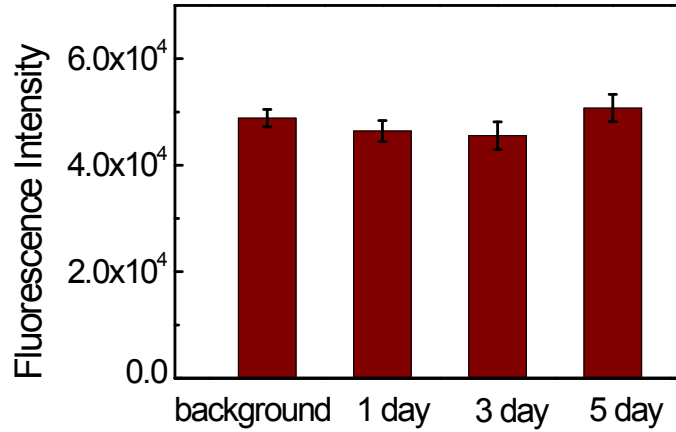


**Fig. S5.** (A) Fluorescence intensity of the unattached ssDNA after mixing ssDNA with different size of AuNPs (5, 13 and 30 nm). (B) MS peak intensities of adenine in the experiments of aptamer mixing with different size of AuNPs (5, 13 and 30 nm) to detect PSA.

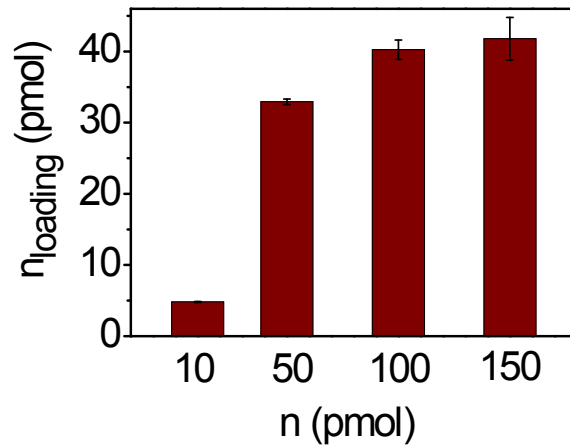


**Fig. S6.** Fluorescence intensity of the ssDNA solution (0.02  $\mu$ M) and the unattached ssDNA after mixing ssDNA (0.02  $\mu$ M) with AuNPs (a), with the addition of 0.1 mM Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, tyrosine and cysteine (b-f), respectively.

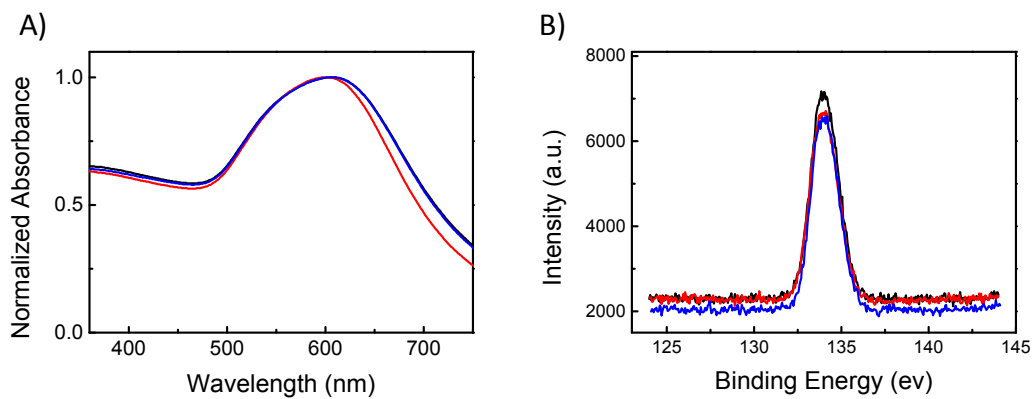




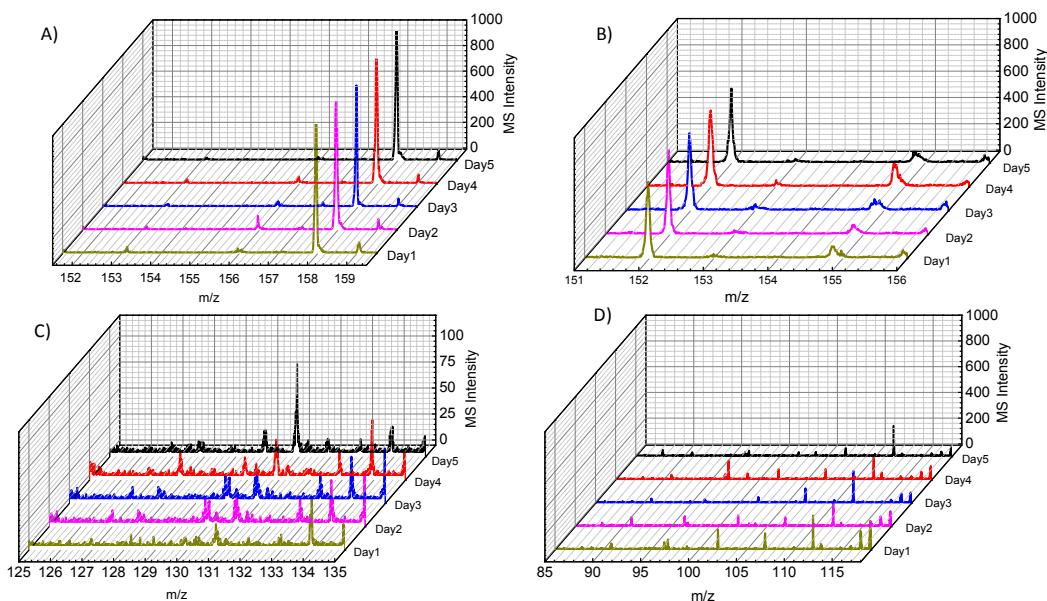
**Fig. S7.** The stability of aptamer-AuNPs. The system of aptamer-AuNPs was kept after moving free aptamers for 1, 3 and 5 days. Then, the free aptamers were measured by fluorescence spectrometer.



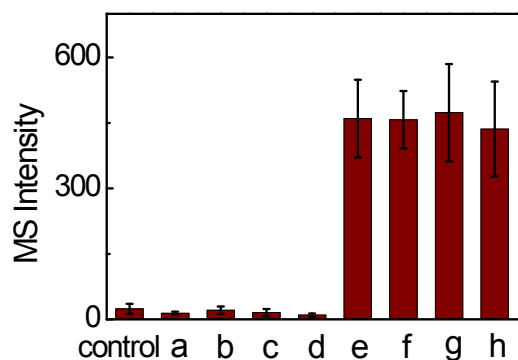
**Fig. S8.** The loading quantity ( $n_{\text{loading}}$ ) of the ssDNA absorbed onto AuNPs.



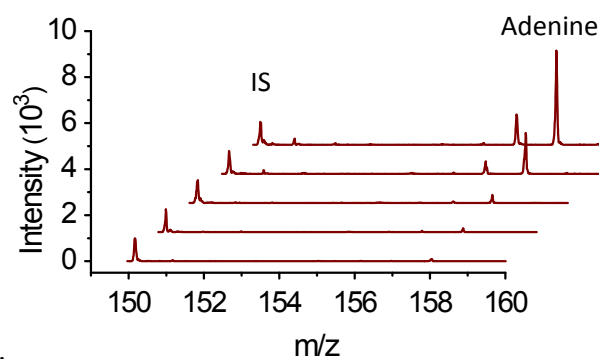
**Fig. S9.** The absorption (A) and the XPS P 2p spectra (B) of Apt<sub>PSA</sub>/AuNPs complex (black line), and with the addition of tamm-horsfall protein (red line) and albumin (blue line), respectively.



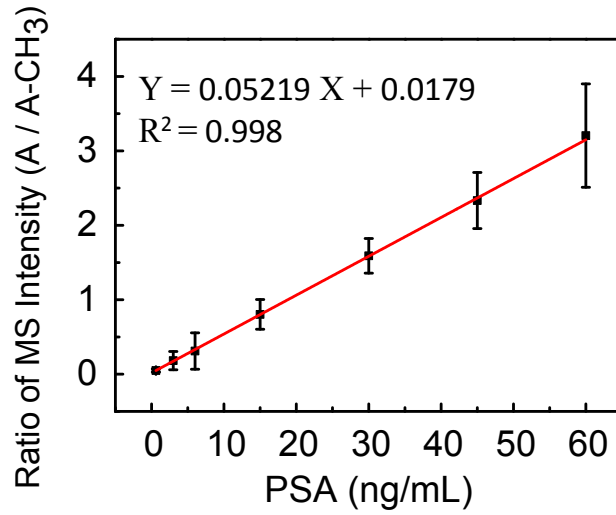
**Fig. S10.** The mass spectrum replicates of the potential tags were performed on five different days. Adenine (A) guanine (B) thymine (C) and cytosine (D) was measured after adding PSA target into the mixture of Apt<sub>PSA</sub>/AuNPs with mass tags.



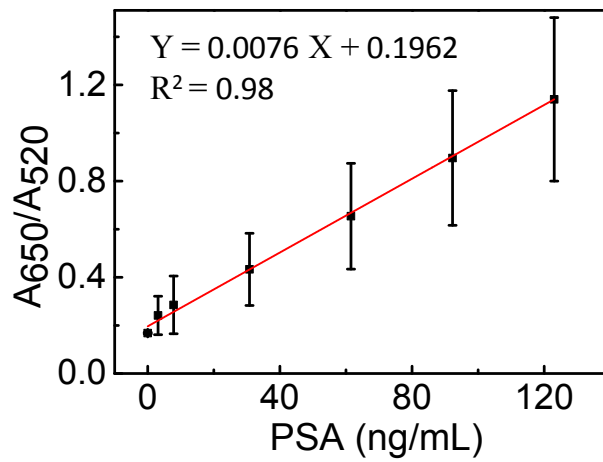
**Fig. S11.** Specificity tests for PSA. MS peak intensities for adenine at 158.0 m/z in control experiments without adding any protein, with the addition of FBA (a), TF (b), IgG (c), HSA (d), PSA (e), the mixture of PSA and FBA (f) and the mixture of PSA and Tamm-Horsefall protein with different concentration (1 and 10 mg/mL) (g and h).



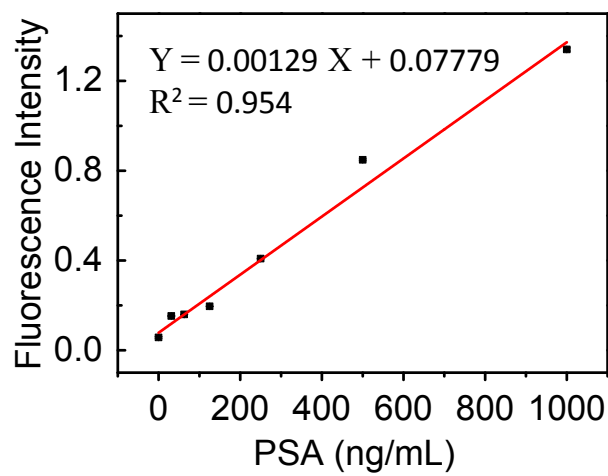
**Fig. S12.** MALDI-MS spectral signals in assays of PSA with varying concentrations (0.6, 3, 6, 30 and 60 ng/mL) based on internal standard (IS).



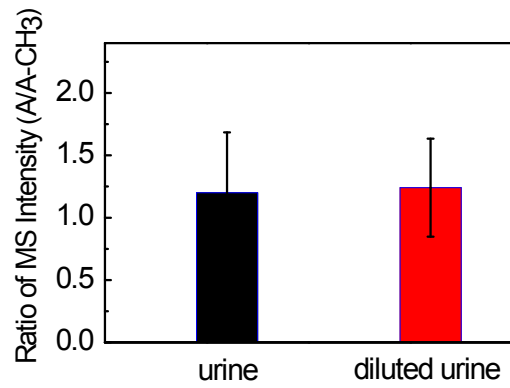
**Fig. S13.** Linear calibration curve of clinical samples detected by MALDI-TOF MS.



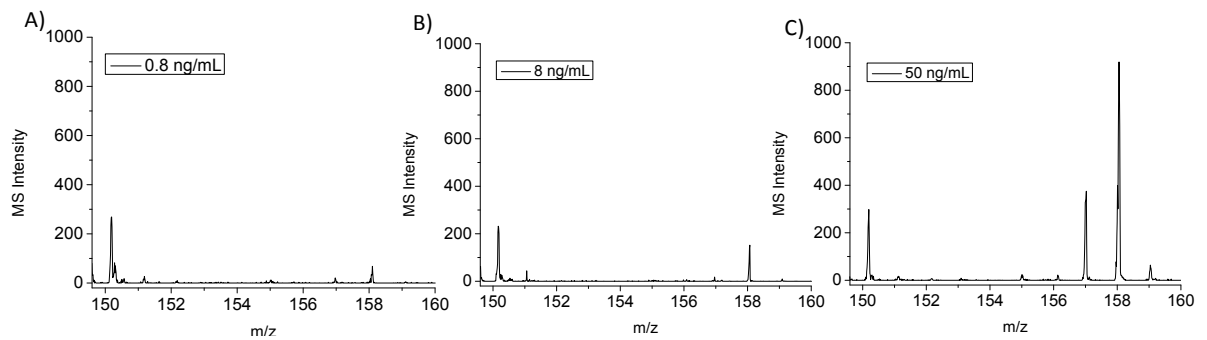
**Fig. S14.** Linear calibration curve of PSA detected by the colorimetric assays.



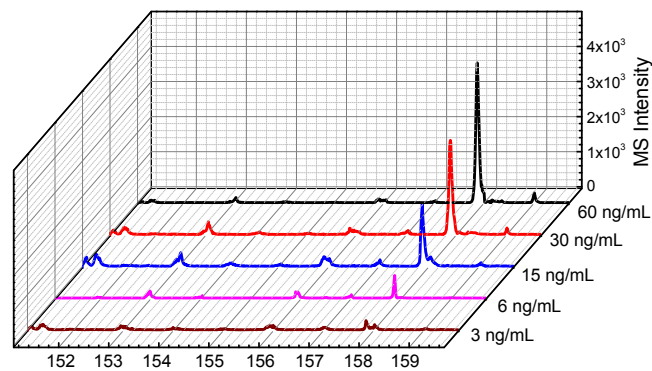
**Fig. S15.** Linear calibration curve of clinical samples detected by ELISA.



**Fig. S16.** The ratio of MS intensity of adenine and internal standard in urine and diluted urine. The protein was excluded from the healthy urine, and the PSA standard was added into the above urine and the diluted urine with the concentration of 20 ng/mL.



**Fig. S17.** The mass spectra of 0.8, 8 and 50 ng/mL PSA (A-C).



**Fig. S18.** MS spectral signal of PSA with various concentrations (3, 6, 15, 30 and 60 ng/mL) in the serum.

**Table S1.** MS approach of PSA calibration solutions.

Concentration (ng/mL)	Average ratio for A/ A-CH <sub>3</sub> in three assays	SD
0	0.007	0.001
0.6	0.049	0.023
3	0.182	0.112
6	0.311	0.232
15	0.803	0.244
30	1.590	0.252
45	2.332	0.376
60	3.205	0.684

**Table S2.** The colorimetric assays of PSA calibration solutions

Concentration (ng/mL)	Fluorescence intensity in three assays	SD
0	0.1680	0.003
3.14	0.2411	0.07
7.84	0.2853	0.11
30.82	0.4331	0.15
61.51	0.6540	0.23
92.27	0.8961	0.28
123.03	1.1400	0.34

**Table S3.** The ELISA of PSA calibration solutions

Concentration (ng/mL)	Fluorescence intensity in three assays	SD
0	0.057	0.001
31.25	0.1525	0.00197
62.5	0.1593	0.00015
125	0.1956	0.001
250	0.4081	0.00151
500	0.8485	0.00419
1000	1.3402	0.00715

**Table S4.** Literature review of PSA detection by different methods.

The detection method	Linear range (ng/mL)	The detection limit (pg/mL)	Ref.
Our approach	0.057-60	57	--
Electrochemistry	--	250	5
Fluorescence microscopy	0.25-100	250	6
Colorimetric detection	--	100	7
LC-MS	1.43-51.43	1430	8

**Table S5.** Recovery test for urine samples

Sample No.	Added concentration (ng/mL)	Concentration obtained with our MS approach (ng/mL) <sup>[a]</sup>	Recovery (%)
1	0	2.37±0.93	--
2	0.8	3.10±0.94	91.25
3	8	9.44±1.13	88.37
4	50	56.81±1.34	108.88

<sup>[a]</sup> the mean ± deviation of three separate measurements

**Table S6.** MS approach of PSA in 5 urine samples.

Sample No.	Concentration obtained with ELISA (ng/mL) <sup>[a]</sup>	Concentration obtained with our MS approach (ng/mL) <sup>[a]</sup>
Health	2.64±1.87	2.37±0.93
Patient1	8.15±2.76	6.90±1.12
Patient2	13.73±1.16	14.27±4.27
Patient3	20.78±0.87	22.50±7.76
Patient4	16.83±3.16	18.57±2.32

<sup>[a]</sup> the mean ± deviation of three separate measurements

## References

1. Y. Jiang, H. Zhao, N. Zhu, Y. Lin, P. Yu, L. Mao, *Angew. Chem. Int. Ed.*, 2008, **47**, 8601-8604.
2. Frens, G. *Nature*, 1973, **241**, 20-22.
3. N. T. Khoa, S. W. Kim, D. H. Yoo, E. J. Kim, S. H. Hahn, *Applied Catalysis A: General*, 2014, **469**, 159-164.
4. J. Duan, M. Yang, Y. Lai, J. Yuan, J. Zhan, *Analytica Chimica Acta*, 2012, **723**, 88-93.
5. J. Okuno, K. Maehashi, K. Kerman and Y. Takamura, *Biosens. Bioelectron.*, 2007, **22**, 2377-2381.
6. K. Kerman, T. Endo, M. Tsukamoto, M. Chikae, Y. Takamura and E. Tamiya, *Talanta*, 2007, **71**, 1494-1499.
7. R. J. Yu, W. Ma, X. Y. Liu, H. Y. Jin, H. X. Han, H. Y. Wang, H. Tian and Y. T. Long, *Theranostics*, 2016, **6**, 1732-1739.
8. Y. Li, Y. Tian, T. Rezai, A. Prakash, M. F. Lopez, D. W. Chan and H. Zhang, *Anal. Chem.* 2011, **83**, 240-245.