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Supporting Information

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## **SERS-Active Silver Nanoparticle Trimers for Sub-**

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## **Attomolar Detection of Alpha Fetoprotein**

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### **5 Material and methods**

#### **6 *Material***

7 All chemicals used in this study were purchased from Sigma-Aldrich. Thiolated  
8 DNA oligonucleotides purified by polyacrylamide gel electrophoresis (PAGE) were  
9 purchased from Shanghai Sangon Biological Engineering Technology & Technology  
10 Co. Ltd. (Shanghai, P.R. China) and suspended in deionized (DI) water to a final  
11 concentration of 100 μM. The DNA sequences are shown in Table S1. Phosphate  
12 Buffer (PB, 5 mM) were prepared by mixing the stock solutions of NaH<sub>2</sub>PO<sub>4</sub> and  
13 Na<sub>2</sub>HPO<sub>4</sub>, and then adjusting the pH to 7.4. Deionized water from a Milli-Q device  
14 (18.2 MΩ, Millipore, Molsheim, France) was used throughout all experiments.

15

#### **16 *Synthesis of Silver Nanoparticles***

17 AgNPs with a diameter of 15 ± 1.7 nm were synthesized according to the  
18 following methods. All glassware were soaked with aqua regia (volume ratio 3:1,  
19 HCl/HNO<sub>3</sub>) and rinsed thoroughly with Millipore-Q water several times. Briefly,  
20 sodium borohydride is 0.6 grams of soluble 20 ml distilled water (ice), and 1% ml of  
21 5 poly (vinyl Pyrrolidone) (PVP) as a stabilizer (protective agent). The solution was  
22 kept in a water-ice bath with high-speed stirring. Next, 5 mL of 1% PVP and 5 mL of  
23 10 mM AgNO<sub>3</sub> were added to the mixture prepared beforehand simultaneously by  
24 two constant-flow pumps at the rate of 30 mL/h. The solution was kept at 80 °C for 3 h

1 to remove the unreacted  $\text{NaBH}_4$ ; Samples prepared yellow. Before use, the AgNPs  
2 solution was centrifuged at 8000 r/min for 10 min and resuspended in 5 mM PB  
3 buffer, the concentration of AgNPs was estimated to be 1 nM based on a previous  
4 method.<sup>1</sup> The prepared Ag NPs solution was stored at 4 °C.

5

### 6 *Self-Assembly of Ag-trimers*

7 In this work, DNA-functionalized Ag NPs were prepared as described in a  
8 previous publication.<sup>2</sup> It is noteworthy that the NP-aptamer were heated at 90 °C for 5  
9 min and then slowly cooled to room temperature after Ag NPs and aptamer have been  
10 mixed.<sup>3</sup> High temperatures made long-chain DNA denatured to an open end and  
11 improved the hybridization of DNA to expected structures in the process of  
12 temperature reduction.<sup>4</sup> The desired Ag-trimers were formed by mixing NP-aptamer  
13 (1.5 mL), NP-DNA1 (2 mL), NP-DNA2 (2 mL) conjugates bearing partially  
14 complementary ssDNA in 5 mM PB buffer containing 50 mM NaCl. Then ATP was  
15 added into the solutions for incubation with a final concentration of 10  $\mu\text{M}$ . At last,  
16 AFP target was added and incubated for 1 h at room temperature, the SERS response of  
17 Ag-trimers decreased rely on the reaction between AFP-aptamer and AFP.

18

### 19 *Fabrication of Ag NPs trimerized sensors*

20 For AFP detection, the SERS sensors were immersed in 5 mM PB solution (50  
21 mM NaCl, pH=7.4) containing various concentrations of AFP and incubated for 30  
22 min. The SERS sensors were then rinsed with 5 mM PB. The final concentrations of  
23 the biomarkers were 0.2 aM, 0.5 aM, 1 aM, 2 aM, 5 aM, 10 aM, 20 aM. A LabRam-  
24 HR800 Micro-Raman spectrometer with Lab-spec 5.0 software attached to a liquid  
25 cell was used. The slit and pinhole were set at 100 and 400  $\mu\text{m}$ , respectively, in the

1 confocal configuration, with a holographic grating (600 g/mm) and an air-cooled He-  
2 Ne laser giving 633nm excitation with a power of ~8 mW. The Raman spectra were  
3 acquired from the substrates for an accumulation time of 15s.

4

#### 5 ***Multiplexed encoded Ag pyramids SERS Assay in serum***

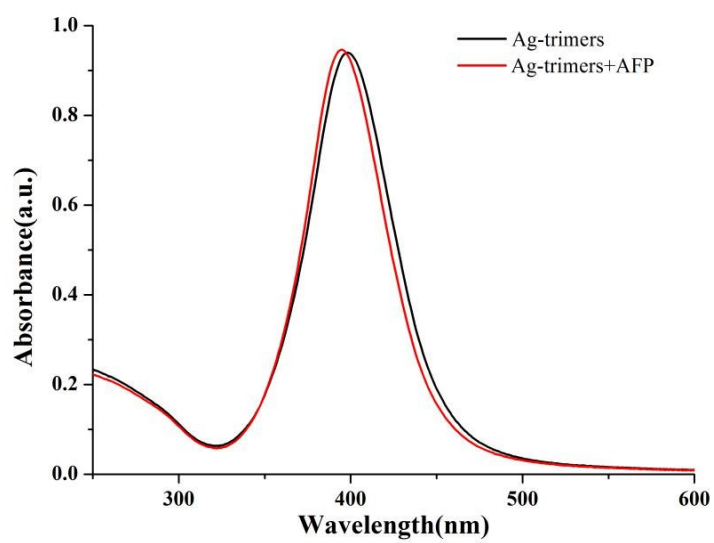
6 For detection of AFP in serum samples, the SERS sensors were immersed in 5mM  
7 PB solution (50 mM NaCl, pH=7.4), serum samples (dilution by  $10^8$ ) were added to  
8 the solution for 30 min reaction, and SERS spectra of the samples were then acquired.  
9 It should be noted that the SERS spectra of serum samples in this study was measured  
10 five times. And the total time for the real serum samples from the patient sample  
11 collection to the concentration results was about 3.5h.

12

#### 13 ***Characterization***

14 TEM images were obtained using a transmission electron microscope (JEOL  
15 JEM-2100) operating at an acceleration voltage of 200 kV. The TEM samples were  
16 prepared by dropping an aqueous dispersion of the sample onto a carbon-coated  
17 copper grid, and then slowly removing the excess sample with filter paper after 5  
18 min. UV/Vis spectra were measured using a UNICO 2100 PC UV/Vis  
19 spectrophotometer. The DLS size was characterized by a Zetasizer Nano ZS system  
20 (Malvern) with a 633 nm laser.

21

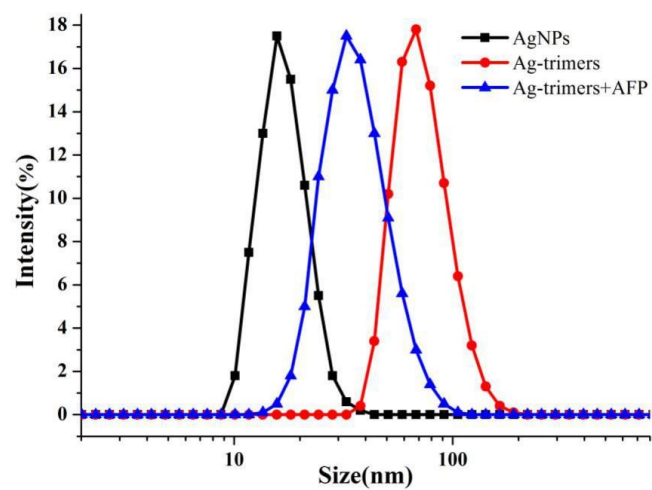


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2 **Figure S1** UV-Vis spectra of Ag-trimers in the absence and that in the presence of 5

3 aM AFP.

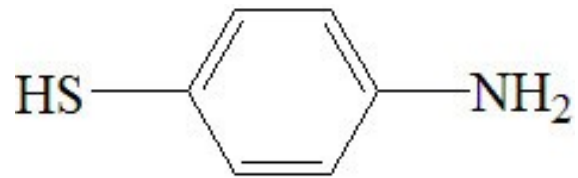
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2 **Figure S2** DLS of Ag-trimers in the absence/presence of AFP (5 aM).

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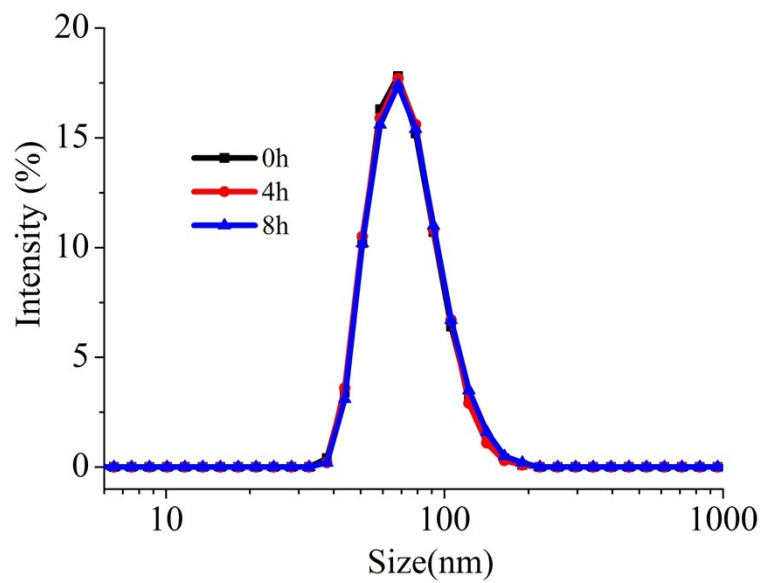
4-ATP

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2 **Figure S3** Raman reporter molecules used in this study: 4-mercaptophenylacetic  
3 acid (4-ATP).

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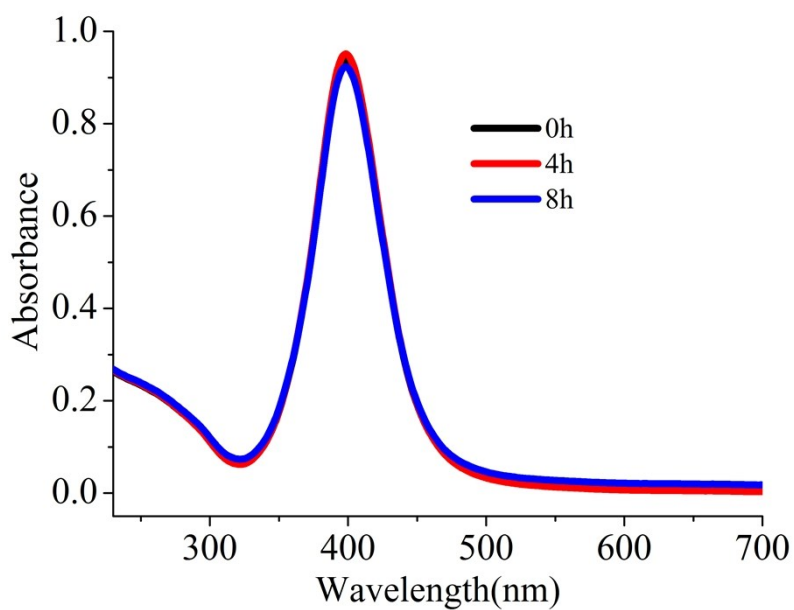
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7 **Figure S4** DLS of Ag-trimers in suspension for different time with the absence of  
8 AFP target.

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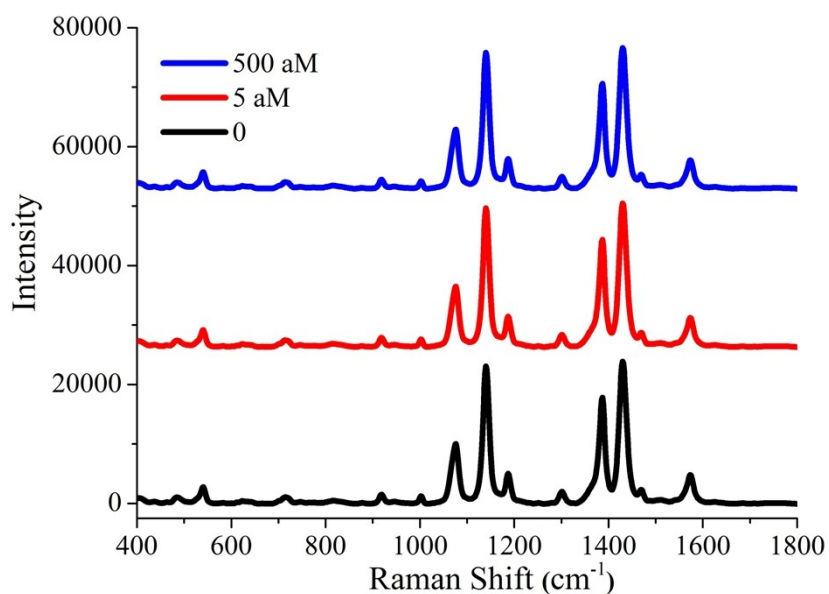


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2 **Figure S5** UV-Vis spectra of Ag-trimers in suspension for different time with the  
 3 absence of AFP target.

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7 **Figure S6** SERS spectra of Ag-trimers for different concentration of AFP target,  
 8 where the trimers were prepared using a DNA sequence that was not able to recognize  
 9 the the target AFP protein.

10

1 **Table S1** DNA sequences for self-assembled trimers and applied in AFP detection.

Types	Sequences
	5'-HS-
Aptamers	GGCAGGAAGACAAACAGGACCGGGTTGTGTGGGGTTTTAAGAG CGTCGCCTGTGTGTGGTCTGTGGTGCTGT-3'
DNA1	5'-HS-CCGGTCCTGTTTGTCTTCCT-3'
DNA2	5'-HS-GGCGACGCTCTTAAAACCCCA-3'
Control	5'-HS-TTTGACTGGAGGACTATGCACATTACGGCTCTCAGTATC
DNA S0	GCAAGGCCTCAGAACCAAGAATCGGTAAGTCGG-3'
Control	5'-ATGTGCATAGTCCTCCAGTC-3'
DNA S1	
Control	5'-TGAGGCCTTGCGATACTGAGA-3'
DNA S2	

2

3



1 **Table S2** Analysis of AFP using various sensing systems.

Number	Detection Method	LOD of AFP	Ref.
1	ECL	0.29 aM	5
2	ECL	2.90 aM	6
3	Magnetic immunoassay	0.87 fM	7
4	Au@Ag nanorod-based colorimetric sensor	0.43 pM	8
5	fluorescent magnetic nanobeads	0.34 pM	9
6	LSPR	0.348 pM	10
7	HEMTs	0.29 pM	11
8	Quantum dot-based immunochromatography	14.5 aM	12
9	SPR	3.62 fM	13
10	Carbon nanotubes	1.45 fM	14
11	RLS	0.58 pM	15
12	SERS	0.02 pM	16

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## 1 References

- 2 1 W. Haiss, N. T. Thanh, J. Aveyard and D. G. Fernig, *Analytical chemistry*, 2007, **79**,  
3 4215.
- 4 2 W. Yan, L. Xu, C. Xu, W. Ma, H. Kuang, L. Wang and N. A. Kotov, *Journal of the*  
5 *American Chemical Society*, 2012, **134**, 15114.
- 6 3 (a) Q. Wang, H. Wang, C. Lin, J. Sharma, S. Zou and Y. Liu, *Chem. Commun.*, 2009, **46**,  
7 240; (b) Y. Xiao, V. Pavlov, R. Gill, T. Bourenko and I. Willner, *ChemBioChem*, 2004, **5**,  
8 374.
- 9 4 (a) L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas and J. J. Toole, *Nature*, 1992,  
10 **355**, 564; (b) Y. Okahata, M. Kawase, K. Niikura, F. Ohtake, H. Furusawa and Y. Ebara,  
11 *Analytical Chemistry*, 1998, **70**, 1288.
- 12 5 Z. Guo, T. Hao, S. Wang, N. Gan, X. Li and D. Wei, *Electrochemistry Communications*,  
13 2012, **14**, 13.
- 14 6 Z. Guo, T. Hao, J. Duan, S. Wang and D. Wei, *Talanta*, 2012, **89**, 27.
- 15 7 H. Tsai, B. Gao, S. Yang, C. Li and C. B. Fuh, *Journal of nanoparticle research*, 2014,  
16 **16**, 2182.
- 17 8 F. Zhang, J. Zhu, J. Li and J. Zhao, *Journal of Materials Chemistry C*, 2015, **3**, 1841.
- 18 9 K. Terada, T. Tanaka, N. Hanyu, T. Honda and H. Handa, *Int J Anal Bio-Sci Vol*, 2014, **2**,  
19 101.
- 20 10 W. Li, X. Jiang, J. Xue, Z. Zhou and J. Zhou, *Biosensors and Bioelectronics*, 2015, **66**,  
21 590.
- 22 11 K. Ding, C. Wang, B. Zhang, Y. Zhang, M. Guan, L. Cui, Y. Zhang, Y. Zeng, Z. Lin and  
23 F. Huang, *Electron Device Letters, IEEE*, 2014, **35**, 333.
- 24 12 Q. Yang, X. Gong, T. Song, J. Yang, S. Zhu, Y. Li, Y. Cui, Y. Li, B. Zhang and J. Chang,  
25 *Biosensors and Bioelectronics*, 2011, **30**, 145.
- 26 13 J. Zhu, Z. Yu, J.-j. Li and J.-w. Zhao, *Sensors and Actuators B: Chemical*, 2013, **188**, 318.
- 27 14 H. Yang, Z. Li, X. Wei, R. Huang, H. Qi, Q. Gao, C. Li and C. Zhang, *Talanta*, 2013, **111**,  
28 62.
- 29 15 Z. Chen, Y. Lei and X. Chen, *Microchimica Acta*, 2012, **179**, 241.
- 30 16 A. Wang, W. Ruan, W. Song, L. Chen, B. Zhao, Y. M. Jung and X. Wang, *Journal of*  
31 *Raman Spectroscopy*, 2013, **44**, 1649.
- 32