Electronic Supplementary Material (ESI) for Analyst

Metal-Enhanced Fluorescent Detection for Proteins Microarrays Based on Silver Plasmonic Substrate

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Synthesis of silver and gold nanoparticles

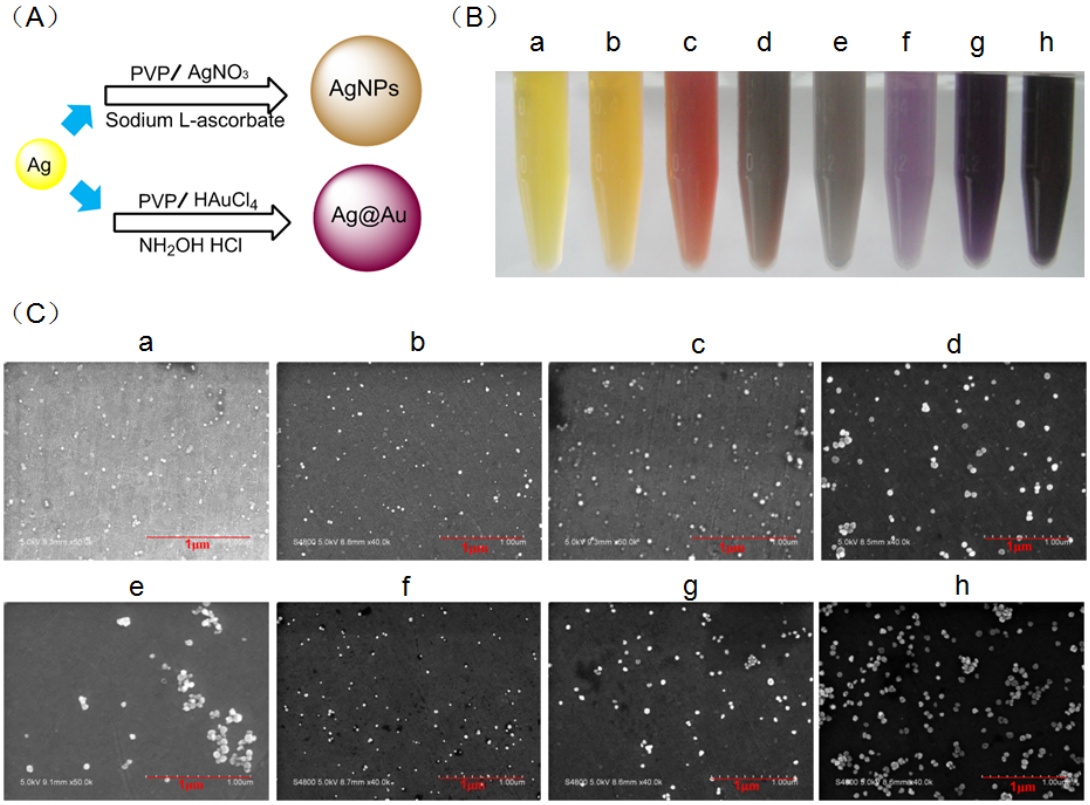

Fig. S1.（A）Schematic illustration of synthesis of silver and Ag@Au nanoparticles with different sizes by seed-mediated growth method. (B) Color photograph of all kinds of silver and Ag@Au. (C) SEM images of oligonucleotides-functionalized nanoparticles a-h.

Table S1. The amount of reagents for synthesis of AgNPs and Ag@Au.

	a	b	c	d	e	f	g	h
AgNPs(mL)	5	5	5	5	5	5	5	5
polyvinylpyrrolidone(1%, mL)	0	5	5	5	5	5	5	5
L-sodium ascorbate(mL, mM)	0	6, 1	6, 4	6, 10	12, 10	0	0	0
silver nitrate(mL, mM)	0	6, 1	6, 4	6, 10	12, 10	0	0	0
hydroxylamine hydrochloride(mL, mM)	0	0	0	0		6, 1	6, 4	6, 10
chloroauric acid(mL, mM)	0	0	0	0		6, 1	6, 4	6, 10

Optimization for functionalization of AgNPs-d

1.5 μg/mL protein PDGF-BB was spotted on the aldehyde-modified slides. After immobilization, blocking and washing, the Tags were added to incubate for 1h. After another washing step, the slide was scanned. **A:**(a)1mL AgNPs-d was modified with SH-oligo(d)A_{24}-TAMRA (25 μL, 10 μM), SH-oligo(d)-A_{15} (25 μL, 10 μM), Apt-PDGF (25 μL, 10 μM), Za-1 (25 μL, 10 μM); (b) with SH-oligo(d)A_{24}-TAMRA (25 μL, 10 μM), SH-oligo(d)-A_{15} (50 μL, 10 μM), Apt-PDGF (25 μL, 10 μM); (c) with SH-oligo(d)A_{24}-TAMRA (50 μL, 10 μM), SH-oligo(d)-A_{15} (25 μL, 10 μM), Apt-PDGF (25 μL, 10 μM); (d) with SH-oligo(d)A_{24}-TAMRA (50 μL, 10 μM), Apt-PDGF (50 μL, 10 μM); B: (a) 1mL AgNPs-d was modified with Apt-PDGF (90 μL, 10 μM) SH-oligo(d)A_{24}-TAMRA (10 μL, 10 μM), (b) with Apt-PDGF (75 μL, 10 μM) SH-oligo(d)A_{24}-TAMRA (25 μL, 10 μM), (c) with Apt-PDGF (50 μL, 10 μM) SH-oligo(d)A_{24}-TAMRA (50 μL, 10 μM), (d) with Apt-PDGF (25 μL, 10 μM) SH-oligo(d)A_{24}-TAMRA (75 μL, 10 μM), (e) with Apt-PDGF (10 μL, 10 μM) SH-oligo(d)A_{24}-TAMRA (90 μL, 10 μM).

Firstly, the influence of other oligonucleotides was investigated and the total amount of oligonucleotides was kept constant. When Za-1 and 5'SH- oligo(d)$A_{15,}$ which had no bio-molecular function, mixed with apt-PDGF and TAMRA to bind with AgNPs-d as detection tags, the results showed that not only Za-1, but also 5'SH- oligo(d)A_{15} would decrease the capture ability of aptamers (Fig. S2-A). When the amount of aptamers and TAMRA was constant, the removal of Za-1 by 5'SH- oligo(d)A_{15} would increase the fluorescence intensity of signal as well as the surrounding background, and the ratio of signal to background was increased. As the length of 5'SH- oligo(d)A_{15} was shorter than Za-1, the side effect was weaker. The amount of aptamers was kept constant, the fluorescence intensity of surrounding background increased dramatically when increasing the amount of TAMRA, resulted in lower ratio of signal to background. The

enhanced surrounding background was due to the nonspecific adsorption of TAMRA on slides. However, when the aptamers and TAMRA were simultaneously increased, the ratio of signal to background was increased to the highest. Therefore, that AgNPs-d functionalized only with aptamers and fluorescent dyes was the best way. Secondly, the ratio of apt-PDGF to TAMRA was also carefully examined, and the results were shown in Fig. S2-B. The optimal ratio for apt-PDGF to TAMRA was 1:1. When the ratio was less than 1:1, the nonspecific absorption of TAMRA resulted in a higher background; and when the ratio was larger than 1:1, the signal to the background were reduced because of the decrease of fluorescent dyes.

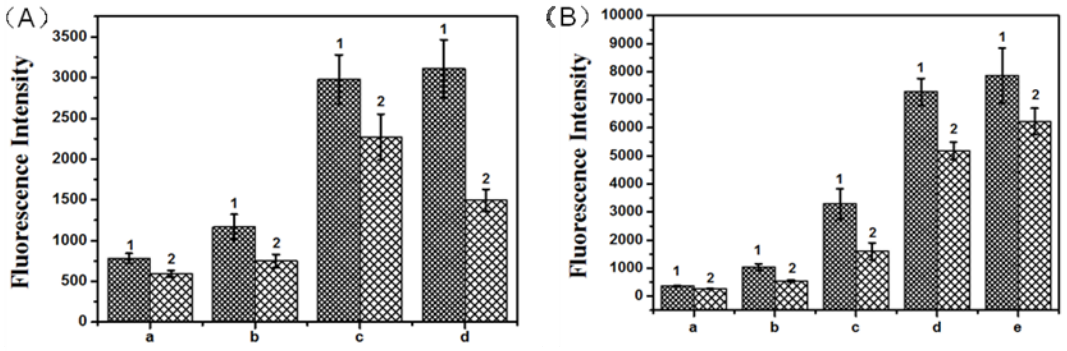

Fig. S2. (A) a: Za-1(25%)+A_{15}(25%)+apt-PDGF(25%)+TAMRA(25%). b: A_{15}(50%)+ apt-PDGF (25%)+TAMRA (25%), c: A_{15}(25%)+ apt-PDGF (25%)+TAMRA(50%), d: apt-PDGF (50%)+ TAMRA (50%).
(B) a: apt-PDGF (90%)+ TAMRA (10%), b: apt-PDGF (75%)+ TAMRA (25%), c: apt-PDGF (50%)+ TAMRA (50%), d: apt-PDGF (25%)+ TAMRA (75%), e: apt-PDGF (10%)+ TAMRA (90%) 1:signal, 2: surrounding background.

Scanning electron microscopy (SEM)

SEM images of Tag-B and Tag-C on different microarrays substrate. Tag-B and Tag-C on antibody substrates were illustrated in Fig. S3 A and D, whereas Fig. S3 B and E indicated the interaction between Tag-B, Tag-C on antibody-AgNPs-a. Furthermore, Tag-B and Tag-C on antibody-AgNPs-d plasmonic microarrays were shown in Fig. S3 C and F. In addition, the SEM images showed that the antibody-AgNPs-a and antibody-AgNPs-d were well immobilized on the slides, which indicated the antibody was successfully bound with AgNPs-a and AgNPs-d.

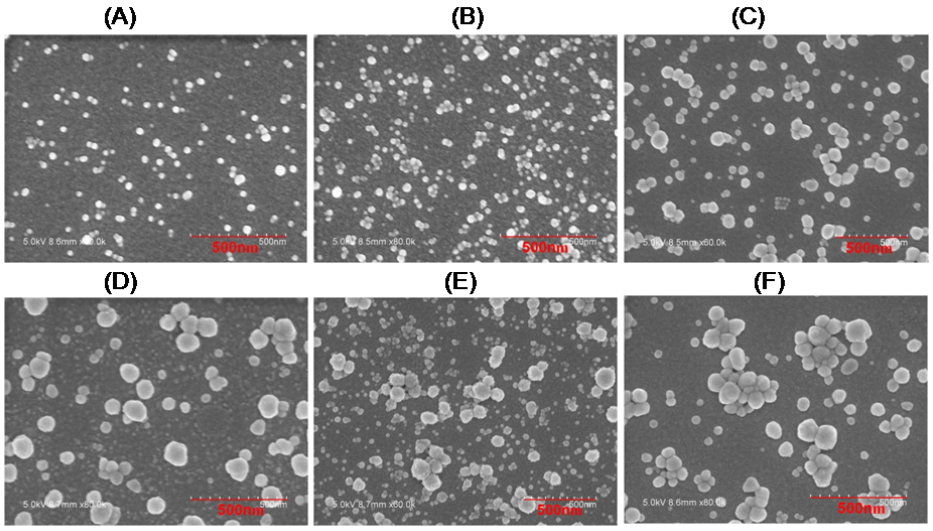

Fig. S3. SEM images of Tag-B on different microarrays substrate, (A) antibody, (B) antibody-AgNPs-a, (C) antibody-AgNPs-d. SEM images of Tag-C on different microarrays substrate, (A) antibody, (B) antibody-AgNPs-a, (C) antibody- AgNPs-d, the concentration of PDGF-BB was 50 ng/mL.

The detecting performance of Tag-B and Tag-C on antibody substrate

The detecting performance of Tag-B and Tag-C on antibody substrate was estimated to be a control, and the results were shown in Fig. S4. For Tag-B, when the PDGF-BB concentration was decreased to 2 ng/mL, the fluorescence intensity was reduced further and the detection limit was near 2 ng/mL. For Tag-C, when the PDGF-BB concentration was decreased to 0.4 ng/mL, the fluorescence intensity was much lower and the detection limit was near 0.4 ng/mL.

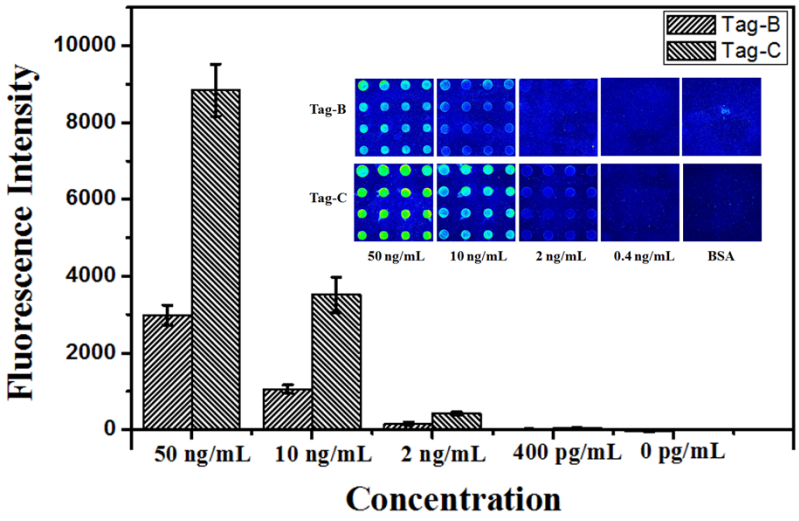

Fig. S4. Fluorescence intensity and images of Tag-B and Tag-C on antibody substrate.