Electronic Supplementary Information (ESI) for Chemical Communications

3D ordered silver nanoshells silica photonic crystal beads for multiplex encoded SERS bioassay

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Experimental

Chemicals and Materials. Human CEA and AFP, a pair of mouse monoclonal anti-human CEA antibodies (D3C, M5B) and a pair of mouse monoclonal anti-human AFP (102K7, 9K5) antibodies were purchased from Beijing Key-bio Biotech Co., Ltd (Beijing, China). 3-Mercaptopropyl trimethoxysilane (MPTS, 95%), 4-mercaptobenzoic acid (4-MBA), butylamine and poly(vinyl pyrrolidone) (PVP, MW=50000) were purchased from Sigma-Aldrich (St. Louis, MO, USA.). Phosphate sodium buffer solution (PBS, 0.1 mM, pH 7.4) was self-prepared. 0.05% Tween-20 was spiked into 0.1 M pH 7.4 PBS as wash buffer (PBST). Milli-Q (Millipore, Bedford, MA) water was used for all experiment. All other reagents were of the best grade available and used as received.

Instrumentation. Scanning electron micrographs (SEM) were obtained by field emitting scanning electron microscopy (FESEM, S-4800, Japan) equipped with an energy-dispersive X-ray spectrometry (EDS), Transmission electron micrographs (TEM) were obtained with a Philips Tecnai-12 electron microscope (Holland) at an acceleration voltage of 120 kV. X-ray photoelectron spectroscopic (XPS) spectrum analysis was measured with an ESCALAB 250Xi spectrometer (USA). A fiber-optic

spectrometer (Ocean Optics, USB4000, USA) was used to record the reflection spectra. Raman measurements were performed with laser Raman spectrometer (Renishaw in via). A He-Ne laser operating at λ=532 nm was used as the excitation source with a 0.5 mW laser power. All Raman spectra were obtained with an exposure time of 20 s.

Synthesis of SPCBs. Various sizes of silica particles (200-300 nm in diameter) were prepared by well-known Stöber method.[S1] The monodisperse SPCBs (average size was about 280 μm) were fabricated by a microfluidic device.[S2] Firstly, monodisperse silica particles were dispersed in water and the suspension was used as the aqueous phase. The oil phase was composed of poly(dimethylsiloxane) and poly-(1,1,1-trifluoropropylmethylsiloxane) (7:3 in volume). Then the oil phase and aqueous suspension were simultaneously injected into the PTFE tube at the speed of 30 and 1 mL/h, respectively. The aqueous suspension was broken into droplets by the oil flows at the needle tip. The formed suspension droplets were delivered by the oil flow into the collection container, which was filled with the silicon oil. Next, the collection container was transfered to an electrothermal oven and maintained at 60 °C for 12 h. At this time, the silica nanoparticles could self-assemble into ordered lattices during the water was evaporated from the suspension droplets. After this solidification step, the fabricated SPCBs were thoroughly washed with hexane to remove the silicon oil.

Preparation of Ag-SPCBs. The SPCBs (~100 beads) were firstly treated with piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 6 hours in order to form abundant OH group on the surface of SPCBs. The SPCBs was then dispersed into 90 mL of absolute ethanol containing water (1:1 v/v), and gently stirred for 10 min at 25 °C in the nitrogen atmosphere. After 25 mL methanol containing 300 μL of MPTS and 750 μL of ammonium hydroxide was added to the mixed solution, and stirred for 6h at 60 °C under the protection of nitrogen, the SH-modified SPCBs were obtained and then washed with methanol and water several times. Next, the Ag-SPCBs were prepared as the previous methods with some modifications.[S3] The SH-modified SPCBs (~100 beads) were dispersed in 25 mL of $AgNO_3$ solution (3.5 mM in methanol) under the gentle stirring. A 50 μL aliquot of butylamine was then rapidly

added into the mixture, and stirred for 3h at 50 °C. The resulting Ag-SPCBs were finally washed with ethanol several times.

Immobilization of antibodies onto Ag-SPCBs. The prepared Ag-SPCBs (~100 spheres) were firstly dispersed in 1 mL of CEA antibody (D3C, 0.5 mg/mL in PBS) or AFP antibody (102K7, 0.5 mg/mL in PBS). After reaction for 2 h at room temperature under shaking, the mixture kept at 4°C overnight. Next, the beads were washed with PBS for three times, and then blocked by 1% BSA in PBS for 2 h at room temperature and finally kept overnight at 4°C. Finally, antibody immobilized Ag-SPCBs were washed carefully with PBS and stored in PBS at 4 °C.

Preparation of SERS signal-amplified probe (4-MBA/antibody/AgNPs). AgNPs were firstly prepared with a microwave method. In brief, the $AgNO_3$ (50 mg) were dissolved in 20 mL of ethylene glycol containing 1.5 g of PVP, and then placed in a microwave reactor (WBFY-205, CHN) for 6 min at a power of 0.5 KW. The resultant AgNPs solution were centrifuged at 7000 rpm for 10 min, washed with water several times and finally dispersed in 5 mL PBS. The size of synthesized AgNPs is about 40 nm (Fig. S3). To prepare SERS signal-amplified probe, 200 μL of Raman label (4-MBA, 16 mM) was added to 1 mL of AgNPs solution, and mixed mildly for 1 h at room temperature. Then, 1 mL of CEA antibody (M5B, 0.5 mg/mL) or AFP antibody (9K5, 0.5 mg/mL) was added to the above-mentioned solution, shaken gently for 2 h at room temperature and finally kept overnight at 4 °C. Unreacted Raman label and antibodies were washed out by twice centrifuging at 7000 rpm for 10 min. The nonspecific sites on the AgNPs surface were blocked using 100 μL 1% BSA in PBS for 2h at room temperature and kept overnight at 4 °C. Afterwards, the mixture was centrifuged at 7000 rpm for 10 min to remove the excessive BSA. The obtained 4-MBA/CEA antibody/AgNPs or 4-MBA/AFP antibody/AgNPs probes were redispersed in 2 mL PBS, and stored at 4 °C until use.

Multiplex SERS detection of tumor markers. For multiplexed detection of tumor markers, two kinds of encoded Ag-SPCBs were used to immobilize CEA and AFP antibodies, respectively. These two kinds of beads were added to a mixture of AFP and CEA in a test tube and incubated for 1 h at room temperature under shaking. Then,

the beads were washed with PBS for three times. Next, SERS signal-amplified probes for CEA and AFP were added to the test tube and reacted at room temperature for 1 h. After washing with PBST for three times, the beads were transferred onto the glass slide. By switching the beam path, Raman spectra and reflection spectra of two kinds of encoded beads were recorded. Based on code information for CEA and AFP, the Raman signals for two tumor markers could be distinguished conveniently.

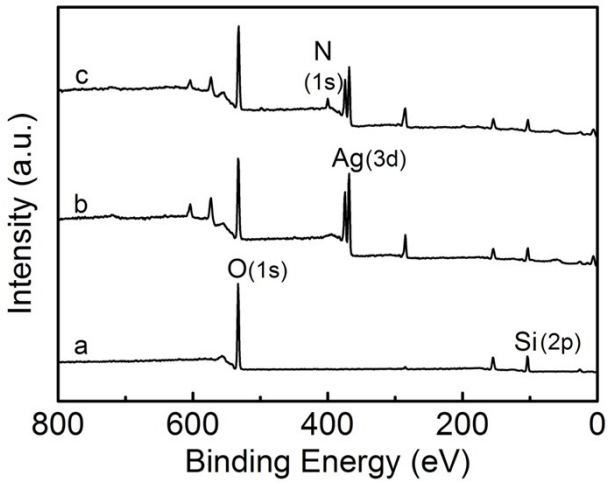

Fig. S1 XPS spectra of SPCBs (a), Ag-SPCBs (b), and antibody immobilized Ag-SPCBs (c).

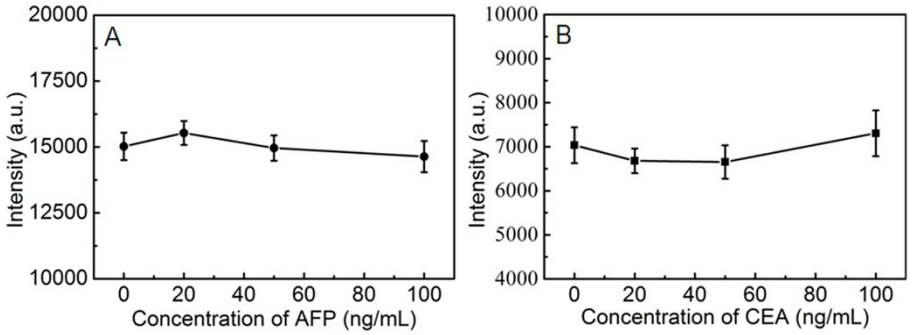

Fig. S2 Cross-reactivity of another analyte to CEA (A) and AFP (B) antibodies in the presence of 20 ng/mL AFP and CEA, respectively (n = 5 for each point).

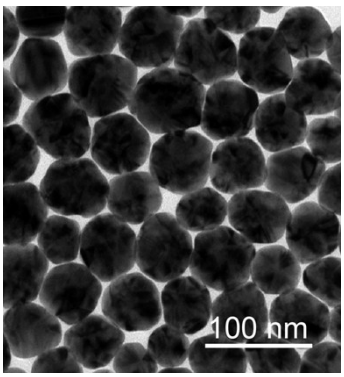

Fig. S3 The TEM image of the synthesized AgNPs

Table S1 Assay results of clinical serum samples using the proposed and reference methods (ng/mL)

sample	CEA			AFP		
	proposed method	reference method	relative error (%)	proposed method	reference method	relative error (%)
1	0.359	0.393	-8.18	2.27	2.16	5.09
2	1.18	1.25	5.60	2.24	2.44	-8.20
3	1.72	1.78	-3.37	3.71	3.45	7.54
4	3.90	3.79	2.90	3.52	3.69	-4.61
5	206.4	195.9	5.36	652.9	618.3	5.60

References

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