Electronic supplementary information

Multimers-based SERS aptasensor for highly sensitive and homogeneous assay of carcinoembryonic antigen

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Experimental section

Detailed sequences of oligonucleotides

CEA-aptamer: 5'-ATACCAGCTTATTCAATT-3'

Complementary single strand-1 (DNA**1**): 5'-SH-C6-TTTTTTTTTTAATTGAAT-3'

Complementary single strand-2 (DNA**2**): 5'-AAGCTGGTATTTTTTTTTT-C6-SH-3'

SERS mapping process

The colloid of Au@Ag multimers was sucked into a capillary glass tube and fixed onto a glass slide. After focusing, the focus area was mapped with the Raman band of 4-MBA at 1585 cm^{-1}. All SERS mapping data were obtained by using a 633 nm laser with 8 mW power and 10× objective. The collecting time was 5 s with 2 rounds of accumulations and the slit aperture was 50 μm. The mapping area was 247 × 40 μm^2 with a step size of 13 μm (x-axis) and 20 μm (y-axis). In all, 60 Raman spectra were collected for the mapping image.

Synthesis of Au NPs

The Au nanoparticles (Au NPs) with the size of 30 nm were synthesized according to previous method [1] as follows: first, 10 mL of $HAuCl_4$ aqueous solution (0.25 mM) was heated to boiling under magnetic stirring. Then 0.15 mL of trisodium citrate (1%, w/w) was quickly injected into the boiling solution, and the mixed solution kept refluxing for 30 min. When the color of the mixed solution become win red, the solution was cooled down to room temperature under stirring for further use. The Au NPs concentration was ~0.29 nM, which was calculated using Beer's law and the extinction coefficient (ε) (30-nm Au NPs is 3.0×10^9 M^{-1} cm^{-1}).[2] UV-vis absorbance spectrum of Au NPs was shown in Fig. S1A.

Synthesis of Au@Ag NPs

To obtain core-shell Au@Ag NPs, 1.5 mL of ascorbic acid (0.10 M) was first added to the above Au seeds solution. After completely stirring, 3.5 mL of $AgNO_3$ (1.0 mM) was added dropwise into the mixed solution at the rate of one drop per 10 s. When Ag^+ was reduced by ascorbic acid, the Ag shells were formed on the surface of Au seeds, and with the increase of silver shell thickness, the solution color changed from wine red to orange. After the dropping of silver nitrate, the solution was stirred for 30 min, and then filtered through a 0.22 μm Millipore membrane before storage in 4 ℃ refrigerator for further use. According to the initial concentration of Au core and the change of volume, the final concentration of Au@Ag NPs was calculated to be ~0.19 nM based on the assumption that silver ions were completely reduced on the surface of Au cores. The TEM image in Fig. S2 showed the average diameter of Au NPs was ~30 nm and the thickness of Ag shell was ~7 nm. UV-vis absorbance spectrum of Au@Ag NPs was shown in Fig. S1A. In addition, Au@Ag NPs with different thicknesses of Ag layers were prepared as above described method, but the addition of $AgNO_3$ was adjusted from 0 mL to 5.5 mL.

Synthesis of Ag NPs

The Ag nanoparticles (Ag NPs) with the size of 30 nm were synthesized following the procedure reported in our previous publication.[3] Briefly, 20 mg of $AgNO_3$ was dissolved in 50 mL of water, and the solution was heated under magnetic stirring. When it was boiling, 2 mL of freshly prepared trisodium citrate (1%, w/w) was added into the $AgNO_3$ solution, and the mixture was refluxed for 1 h until the solution color became grey green. The resultant Ag colloid was gradually cooled to room temperature under stirring and stored in a refrigerator at 4 ℃ for further use. The Ag NPs concentration was 0.51 nM, which was calculated using Beer's law

and the extinction coe□cient (ε) (30-nm Ag NPs is 2.3×10^{10} M^{-1} cm^{-1}).[2] UV-vis absorbance spectrum of Ag NPs was shown in Fig. S1A.

Calculation of enhancement sensitivities (Es)

The enhancement sensitivities (Es) of individual particles to 4-MBA were evaluated by the equation: Es = $I/(C \times M)$, in which I is the intensity of Raman peak at 1585 cm^{-1} assigned to ν(C-C), C is the concentration of 4-MBA (1×10^{-5} M) and M is the concentrations of particles in Au@Ag multimers, Au@Ag NPs colloid (0.19 nM), Ag NPs colloid (0.51 nM) and Au NPs colloid (0.29 nM). Considering that the unmodified reporter molecule will be lost during the centrifugal process involved in the preparation of self-assembled Au@Ag multimers and the accurate concentration of 4-MBA cannot be calculated, we prepared the self-assembled multimers without Raman reporter, and the concentration of Au@Ag NPs in multimers was calculated to be ~0.14 nM according to the initial concentration of Au@Ag NPs and the change of volume, based on the assumption that no particles lost during centrifugation. SERS spectra of 4-MBA (1×10^{-5} M) in Au@Ag multimers, Au@Ag NPs, Ag NPs and Au NPs were shown in Fig. S7. It can be estimated that the enhancement effect of each particle in Au@Ag multimers is ~6.0-, ~26.8- and ~96.2-fold those of Au@Ag NPs, pure Ag NPs and pure AuNPs.

Optimization of experimental conditions

In order to synthesis self-assembled Au@Ag multimers with high Raman enhancement and capture capacity, the concentration of aptamer and the incubation time between aptamer and MBA-DNAs-Au@Ag NPs were optimized. In Fig. S10A, a series of aptamer with different concentrations are added into the mixture of MBA-DNA**1**-Au@Ag NPs and MBA-DNA**2**-Au@Ag NPs and incubates at 25°C for 2h. Clearly, the Raman intensity rapidly enhances with

the concentration of CEA from 0.01 μM to 0.08 μM, and finally reaches the maximum at the concentration 0.08 μM (Fig. S10B).

For the optimization of incubation time, aptamer with the concentration of 0.08 μM is first added into the mixture of MBA-DNA**1**-Au@Ag NPs and MBA-DNA**2**-Au@Ag NPs, and then incubated at 25°C for different times (0, 20, 40, 60, 80, 100, 120 min, respectively). As observed in Fig. S10C, the Raman intensity obviously increased with prolonged reaction time from 0 min to 60min. After 60 min, the signal intensity is almost constant (Fig. S10D). Thus, aptamer (0.08 μM) and incubation time (60 min) are used in the synthesis of self-assembled Au@Ag multimers.

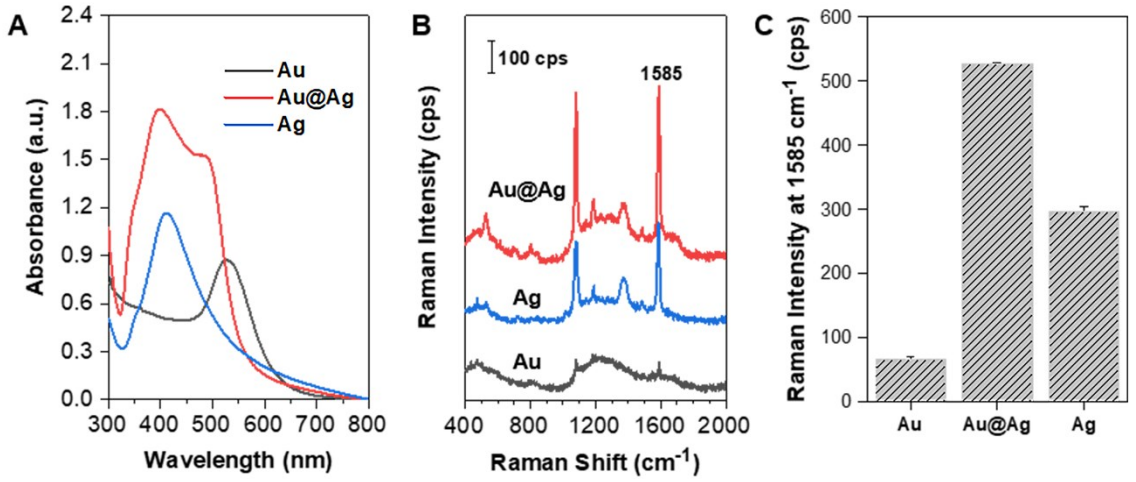


Fig. S1 (A) UV−vis absorption spectra of Ag NPs, Au NPs and Au@Ag NPs. Notably, Ag colloid was diluted 10 times before measuring and the other two colloids kept the original concentrations. (B) SERS spectra of 4-MBA in the colloids of Ag NPs, Au NPs and Au@Ag NPs, respectively. (C) The variation of Raman intensity at 1585 cm^{-1}. The final concentration of 4-MBA is 10 μM.

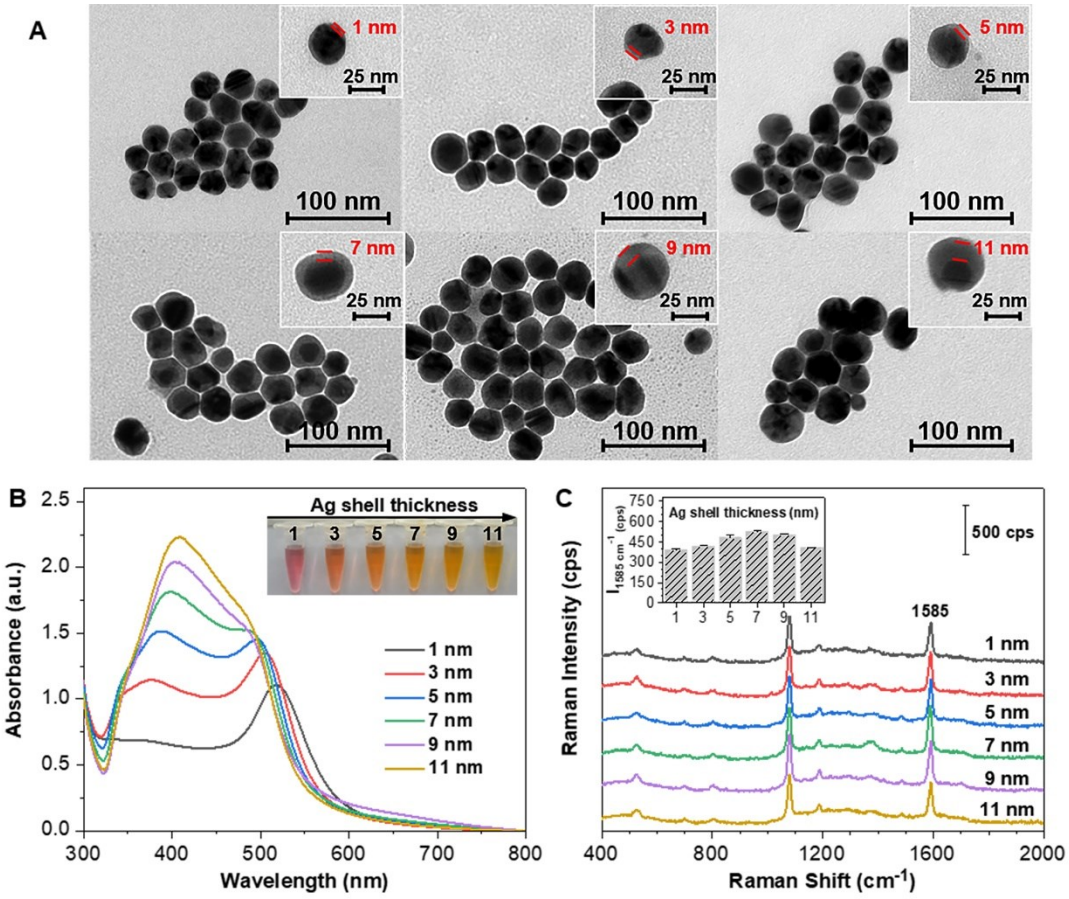


Fig. S2 (A) TEM images of Au@Ag NPs with Ag shell thickness from 1, 3, 5, 7, 9, and 11 nm, respectively. (B) UV–vis absorption spectra of Au@Ag NPs colloid with different Ag shell thicknesses (the inset is the corresponding color images). (C) SERS spectra of 4-MBA in Au@Ag NPs colloid with different Ag shell thicknesses (the inset is the variation of Raman intensity at 1585 cm^{-1}). The final concentration of 4-MBA is 10 μM.

Base sequence of single-strand DNAs with different length of T-base

10T

5'-SH-C6-TTTTTTTTTTAATTGAAT-3' (DNA**1**)

5'-AAGCTGGTATTTTTTTTTT-C6-SH-3' (DNA**2**)

16T

5'-SH-C6-TTTTTTTTTTTTTTTTAATTGAAT-3' (DNA**3**)

5'-AAGCTGGTATTTTTTTTTTTTTTTT-C6-SH-3' (DNA**4**)

32T

5'-SH-C6-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAATTGAAT-3' (DNA**5**)

5'-AAGCTGGTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-C6-SH-3' (DNA**6**)

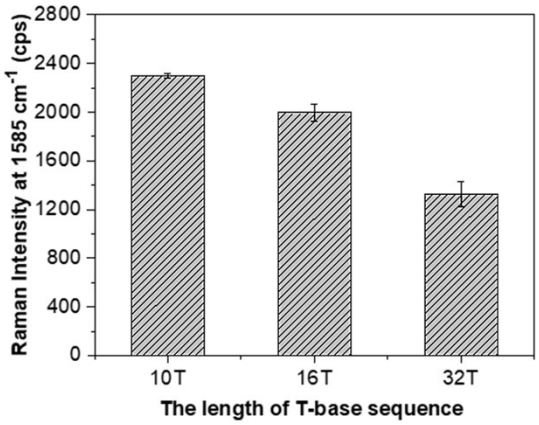


Fig. S3 Variations of 4-MBA Raman intensity (at 1585 cm^{-1}) with the length of T-base sequence.

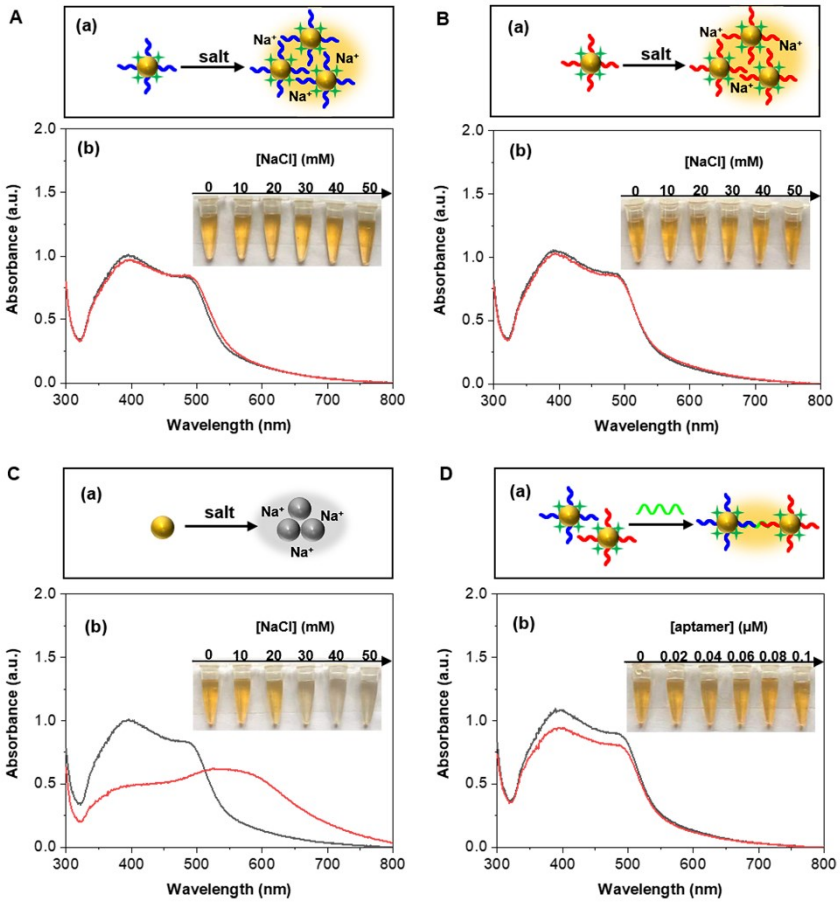
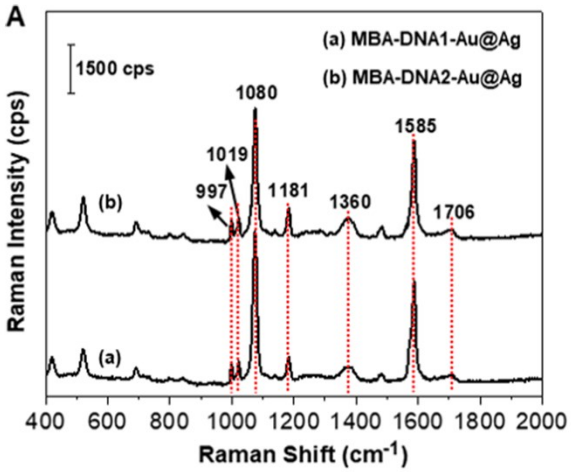


Fig. S4 (A, B and C) UV–vis absorption spectra of MBA-DNAs-Au@Ag NPs and pure Au@Ag NPs in NaCl solution with different concentrations, respectively, black line: 0 mM NaCl; red line: 50mM NaCl (the inset is the corresponding color images). (D) UV–vis absorption spectra of the mixture of MBA-DNA**1**-Au@Ag NPs and MBA-DNA**2**-Au@Ag NPs in aptamer solution with different concentrations, respectively, black line: 0 μM aptamer; red line: 0.1 μM aptamer (the inset is the corresponding color images).



B

Wavenumbers (cm^{-1})	Band assignments
997	$\nu(CC)_{ring}$
1019	$\nu(CC)_{ring}$
1080	ν(CC'+CC)
1132	δ(CH)
1181	δ(CH)
1360	$\nu(COO^-)$
1585	totally symmetric ν (CC)
1706	ν(C=O)

δ: bend or deformation; ν:stretch;
ring : ring breathing mode.

Fig. S5 (A) SERS spectra of 4-MBA in MBA-DNA**1**-Au@Ag NPs (a) and MBA-DNA**2**-Au@Ag NPs (b). (B) The corresponding vibration modes of 4-MBA are recorded from SERS spectra and the peaks are assigned according to references.[4, 5]

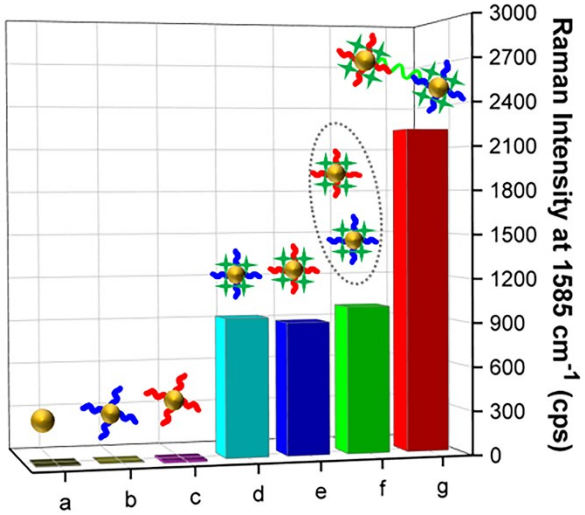


Fig. S6 Variations of intensity (at 1585 cm^{-1}) in the SERS spectra of Au@Ag NPs (a), DNA**1**-Au@Ag NPs (b), DNA**2**-Au@Ag NPs (c), MBA-DNA**1**-Au@Ag NPs (d), MBA-DNA**2**-Au@Ag NPs (e), the mixture of MBA-DNA**1**-Au@Ag NPs and MBA-DNA**2**-Au@Ag NPs (f) and the self-assembled Au@Ag multimers (g).

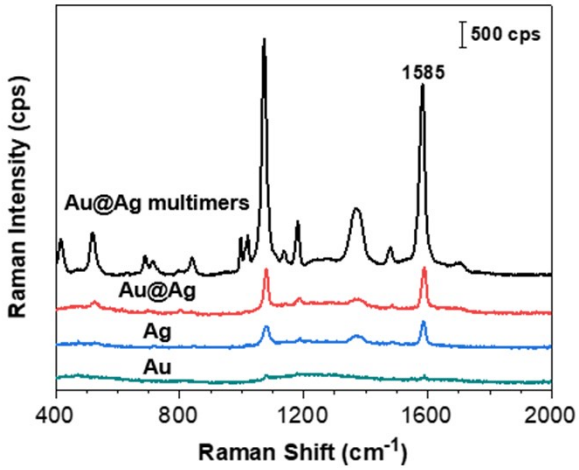


Fig. S7 SERS spectra of 4-MBA (1×10^{-5} M) in Au@Ag multimers, Au@Ag NPs, Ag NPs and Au NPs, respectively.

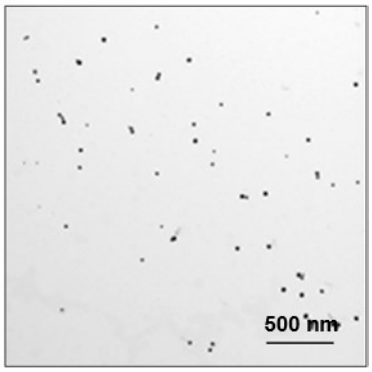


Fig. S8 TEM image of the mixture of MBA-DNA**1**-Au@Ag NPs and MBA-DNA**2**-Au@Ag NPs in the absence of aptamer.

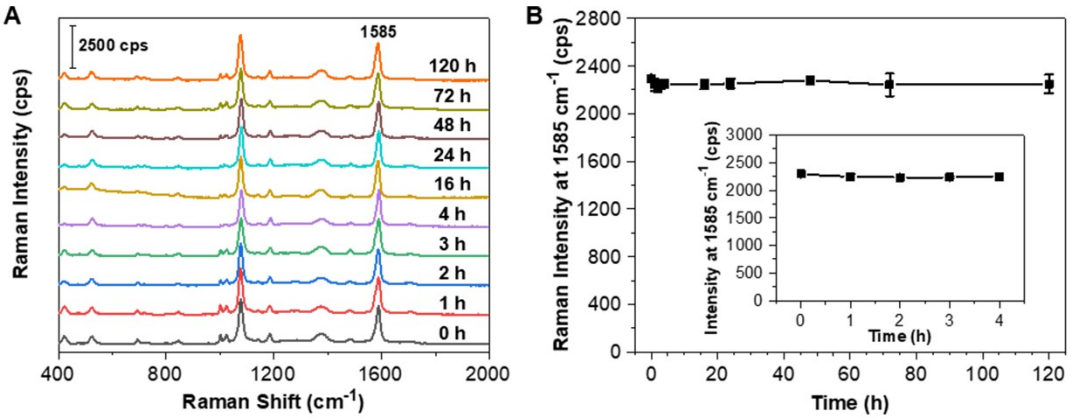


Fig. S9 (A) The time-resolved Raman spectra of Au@Ag multimers. (B) The corresponding Raman intensity of band at 1585 cm^{-1}.

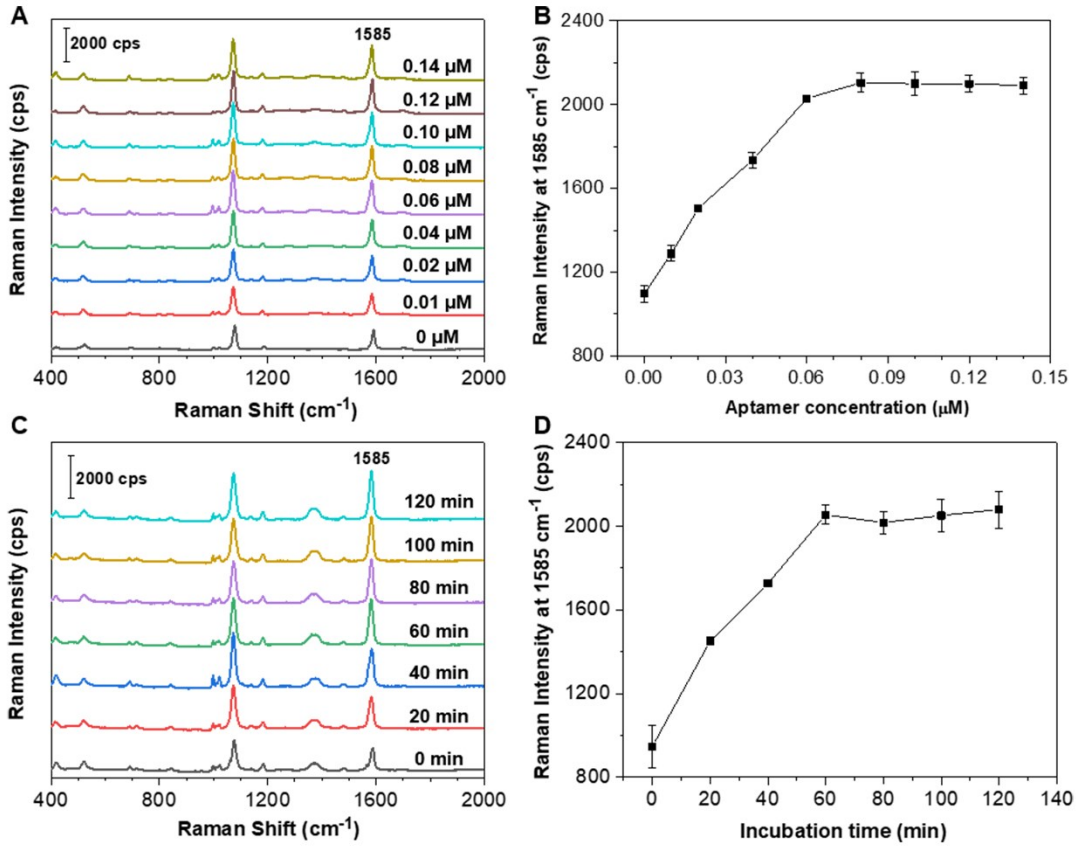


Fig. S10 The effect of (A) aptamer concentration and (C) incubation time on the Raman spectra of Au@Ag multimers. (B and D) The corresponding Raman intensity of band at 1585 cm^{-1} in Fig. S10A and C, respectively. Error bars indicate the standard deviations of three independent measurements.

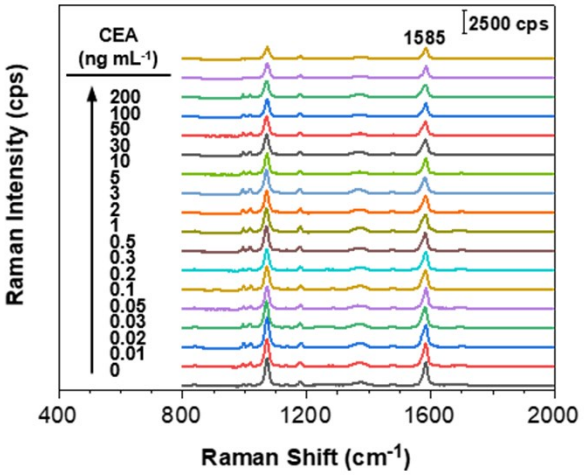


Fig. S11 Evolution of SERS spectra of Au@Ag multimers with the addition of CEA from 0 to 200 ng mL^{-1}.

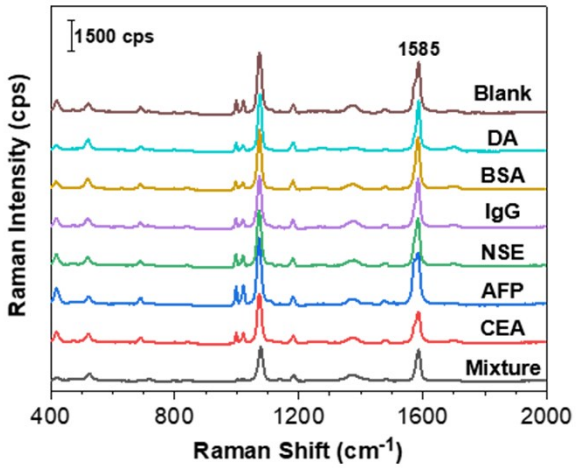


Fig. S12 SERS spectra of Au@Ag multimers for the determination of CEA over other interferons. The concentration of CEA and other interferons are 10 ng mL^{-1} and 10 μg mL^{-1}, respectively.

Table S1 Recovery test (n=5) of CEA in human serum samples.

Sample	Baseline (ng mL^{-1})[a]	Spiked (ng mL^{-1})	Measured (ng mL^{-1})	Recovery (%)	RSD (%)
Normal human Ⅰ	1.52	5	6.38	97.20	3.56
		10	11.76	102.40	3.46
		15	16.57	100.33	2.16
		25	26.80	101.12	3.55
		50	50.76	98.48	4.08
Normal human Ⅱ	4.58	5	9.62	100.80	6.43
		10	14.33	97.50	2.29
		15	19.99	102.73	4.96
		25	29.33	99.00	1.70
		50	57.81	106.46	2.96

[a] The baseline concentrations of CEA from serum samples were detected by the proposed method.

Table S2 Comparison of the methods for CEA detection.

Detection method	Recognition element	Detection time	LOD (pg mL^{-1})	Linearity range (ng mL^{-1})	Ref
Fluorescence	Aptamer	> 1 h	0.8	2×10^{-3} -1×10^{-1}	6
Electrochemistry	Antibody	-	0.0167	5×10^{-5} - 1×10^{2}	7
Colorimetry	Antibody	> 2 h	21.1	5×10^{-2} - 1×10^{2}	8
Resonance light scattering	Antibody	> 20 min	30	1×10^{-1} - 6×10^{1}	9
Love wave	Antibody	-	84	5×10^{-2} - 8×10^{1}	10
Photoelectrochemistry	Antibody	> 30 min	6.12	2×10^{-2} - 5×10^{1}	11
SERS	Antibody	> 4 h	3.01	1×10^{-2} - 1×10^{3}	12
SERS	Antibody	> 2 h	20	5×10^{-2} - 2	13
SERS	Antibody	15 min	10	1×10^{-2} - 5×10^{2}	14
SERS	Antibody& Aptamer	> 2 h	10,000	1×10^{1} - 1×10^{4}	15
SERS	Aptamer	20 min	3.24	1×10^{-2} - 1	This work

Reference in electronic supplementary information

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