Electronic Supplementary Information

Multiplexed detection of serological cancer markers with plasmon-enhanced Raman spectro-immunoassay

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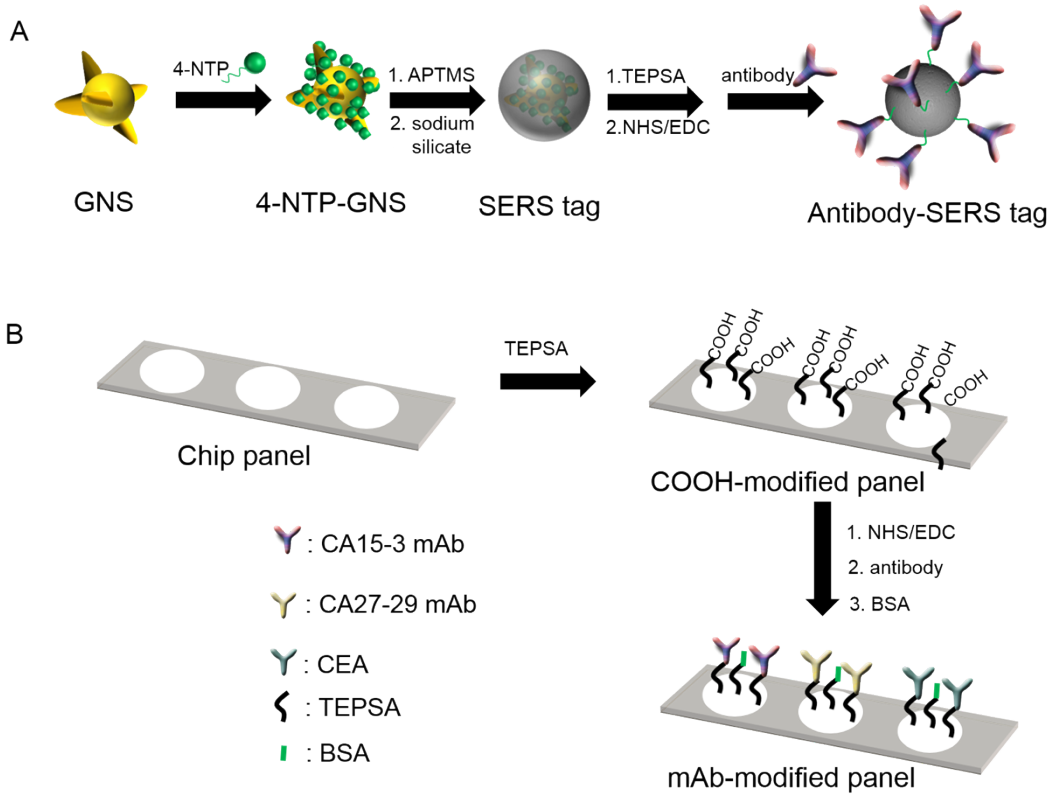


Figure S1. Schematic illustrations of SERS tag preparation and antibody functionalization on SERS tags and chip panel. (A) SERS tag synthesis and its conjugation onto antibodies (CA15-3 mAb, CA27-29 mAb and CEA mAb). (B) SERS assay panel modification with functional antibodies.

Table S1. Peak assignments of Raman and SERS spectra of 4-NTP and SERS tags[1,2]

Raman shift (cm^{-1})	Vibrational assignment
1574	Stretching vibration of phenyl ring
1333	Stretching vibration of N-O
1107	Bending vibration of C-H
1084	Stretching vibration of C-S
855	Wagging vibration of C-H
725	Wagging vibrations of C-H, C-S and C-C

1. S. Hong and X. Li, *J. Nanomater.,* 2013, **49**, 790323.
2. Z. Y. Bao, D. Y. Lei, R. Jiang, X. Liu, J. Dai, J. Wang, H. L. Chan and Y. H. Tsang, *Nanoscale,* 2014, **6**, 9063-9070.

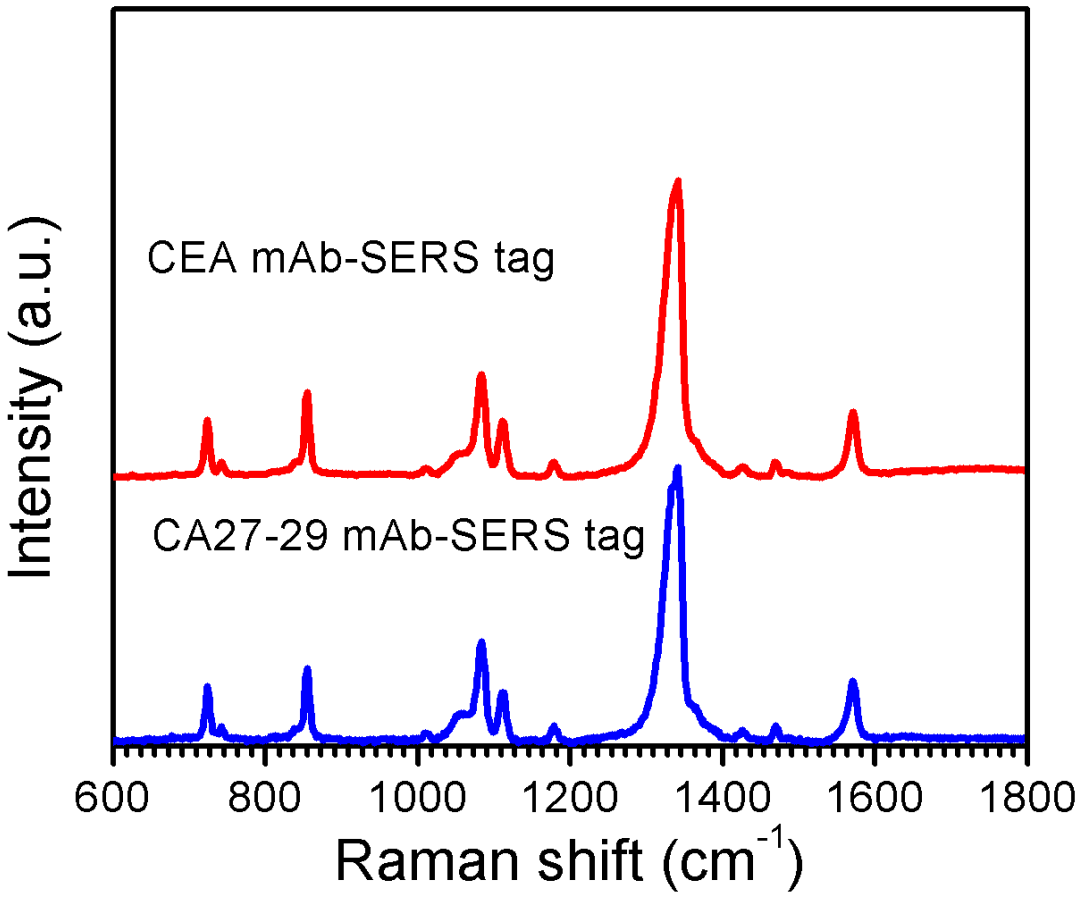


Figure S2. SERS spectra of CA27-29 mAb modified SERS tags and CEA mAb modified SERS tags.

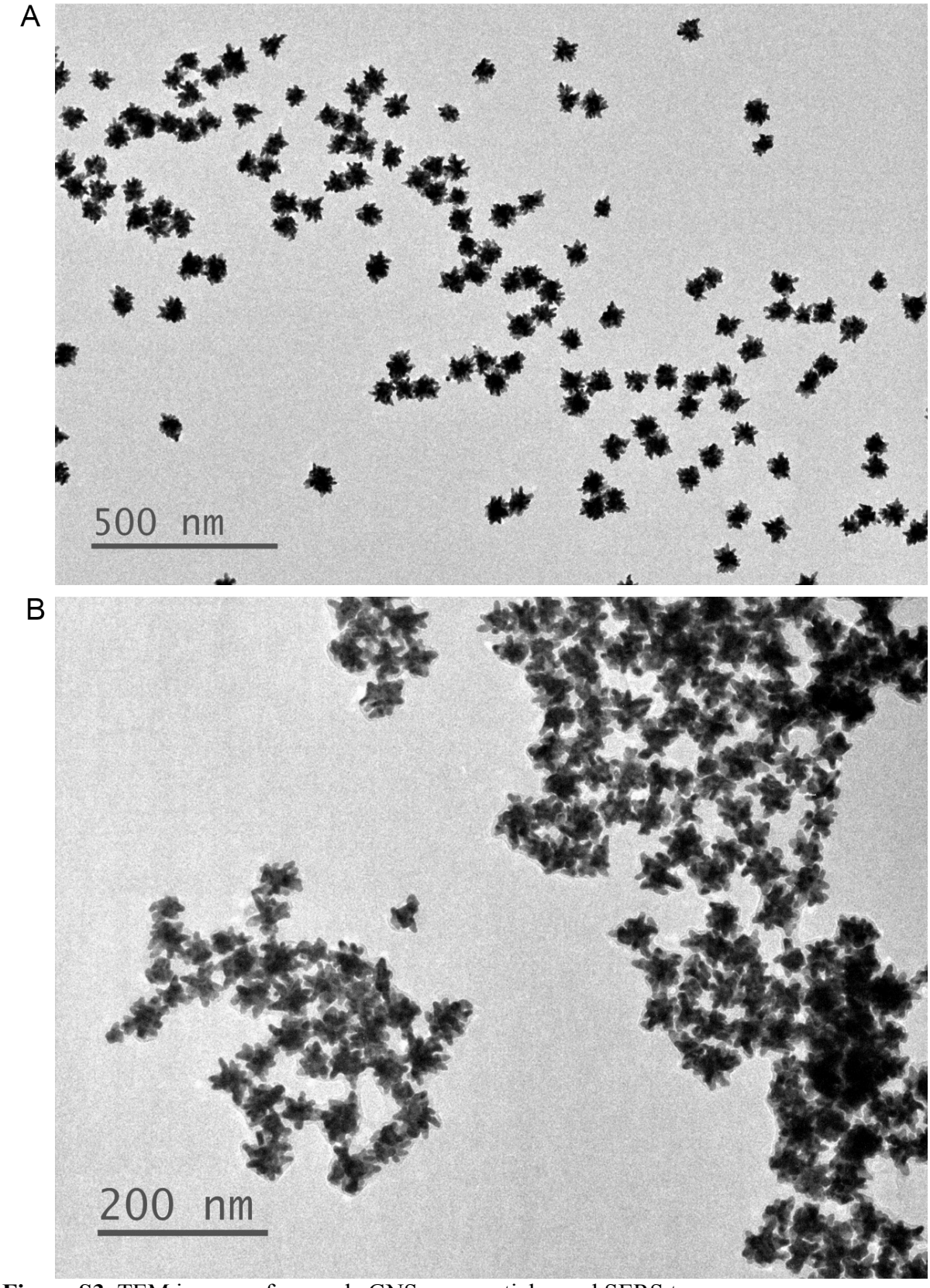


Figure S3. TEM images of as-made GNS nanoparticles and SERS tags.

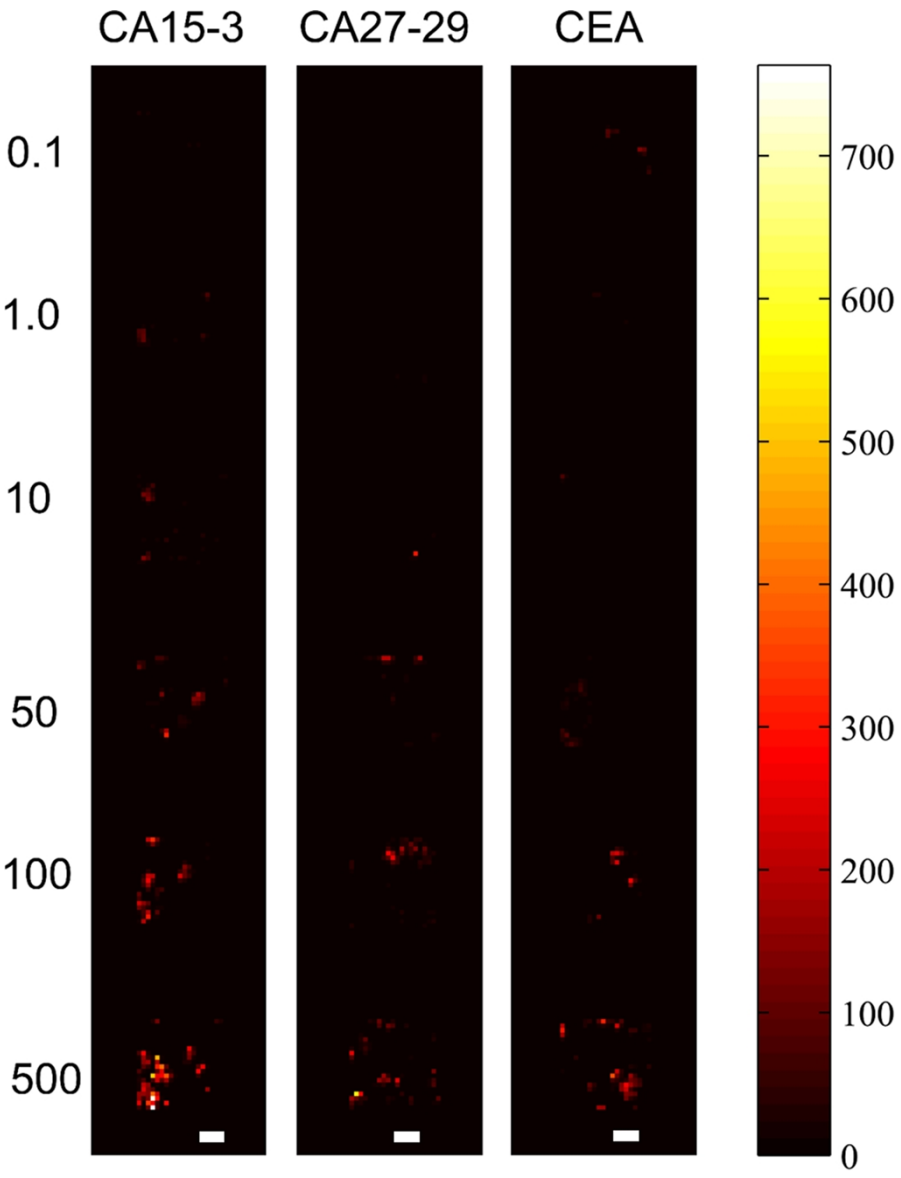


Figure S4. Concentration-dependent SERS assays of CA15-3, CA27-29 and CEA in serum. The concentrations are 0.1, 1.0, 10, 50, 100 and 500 U/mL for CA15-3 and CA27-29, respectively, while the concentrations are 0.1, 1.0, 10, 50, 100 and 500 ng/mL for CEA. Corresponding concentration for each image is shown in the left. Scale bar is 20 μm.

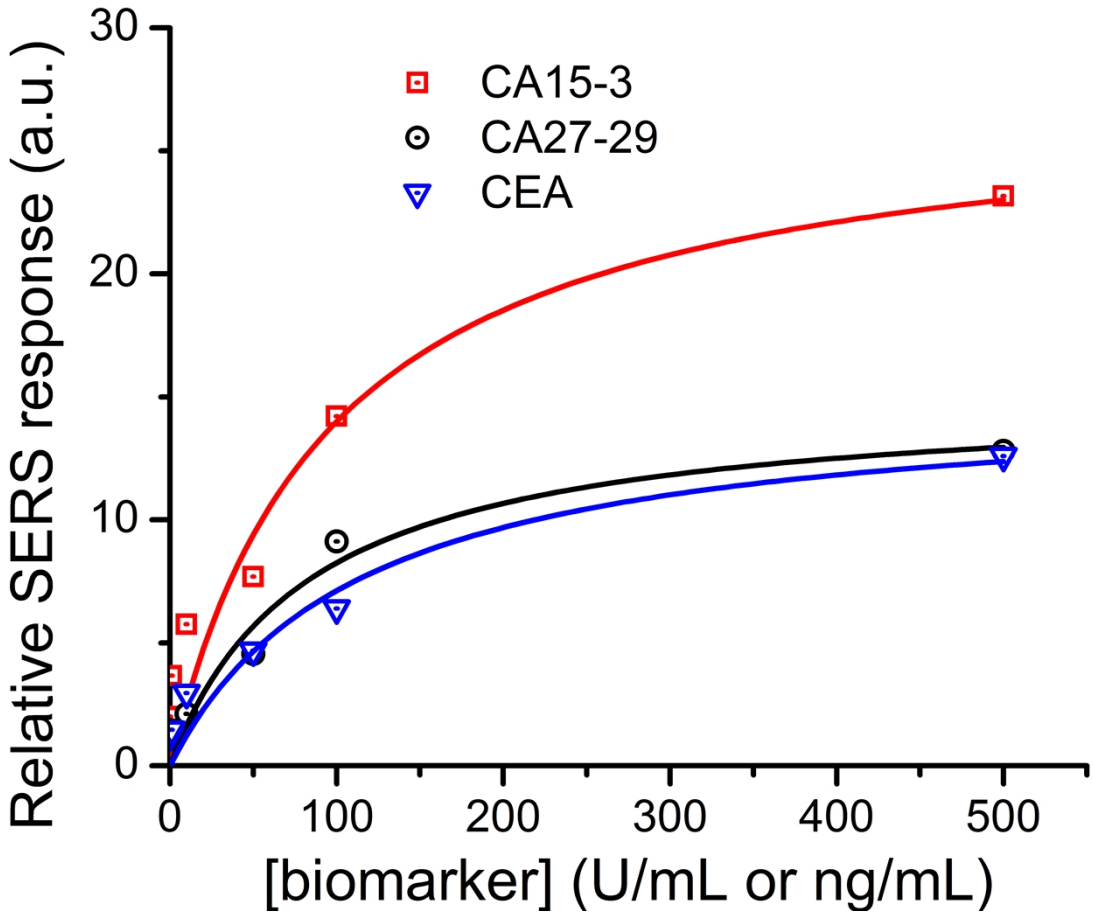


Figure S5. Concentration-dependent SERS assay of CA15-3, CA27-29 and CEA in serum. Fittings of curves are performed using Langmuir isotherms:

$$y = y_0 \bullet \frac{x}{k_d + x}$$

where y is relative SERS response, y_0 is a constant, x is the biomarker concentration, and k_d is the dissociation constant. Thus, we obtain the dissociation constants in sera: 95.9 U/mL for CA15-3, 83.1 U/mL for CA27-29, and 113.2 ng/mL for CEA, respectively.

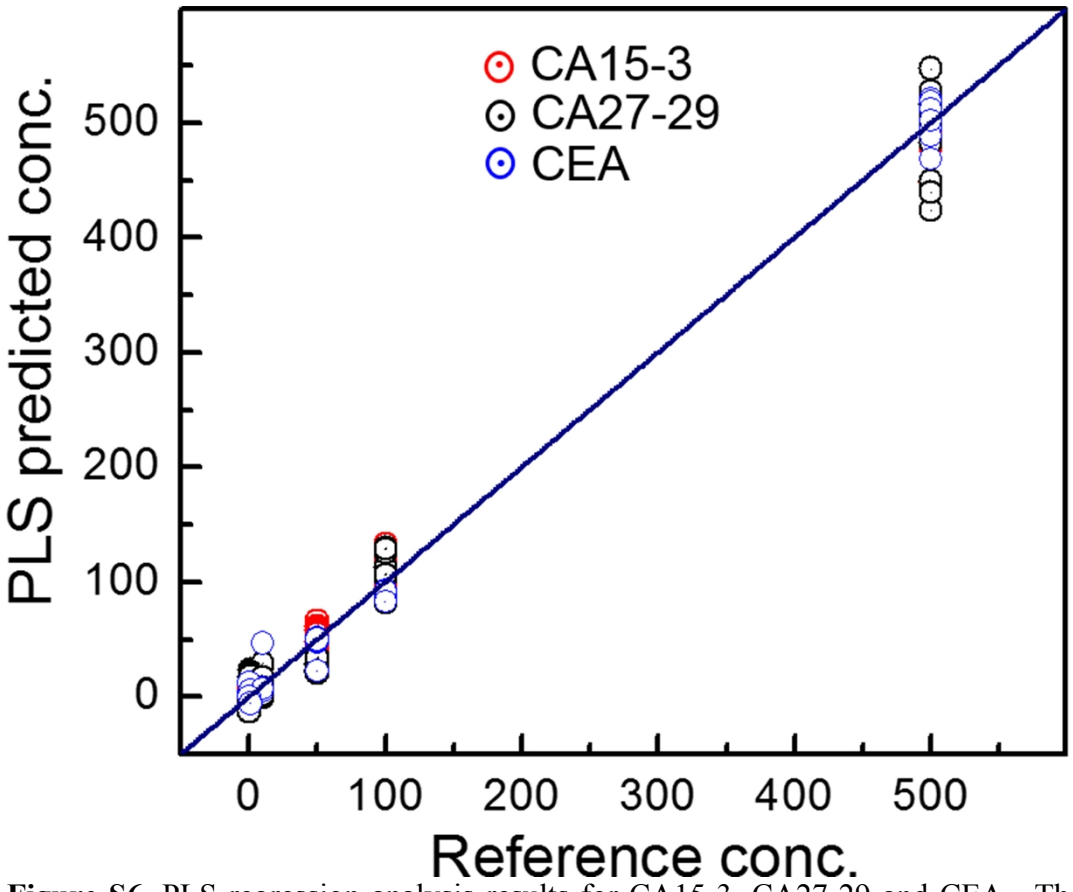
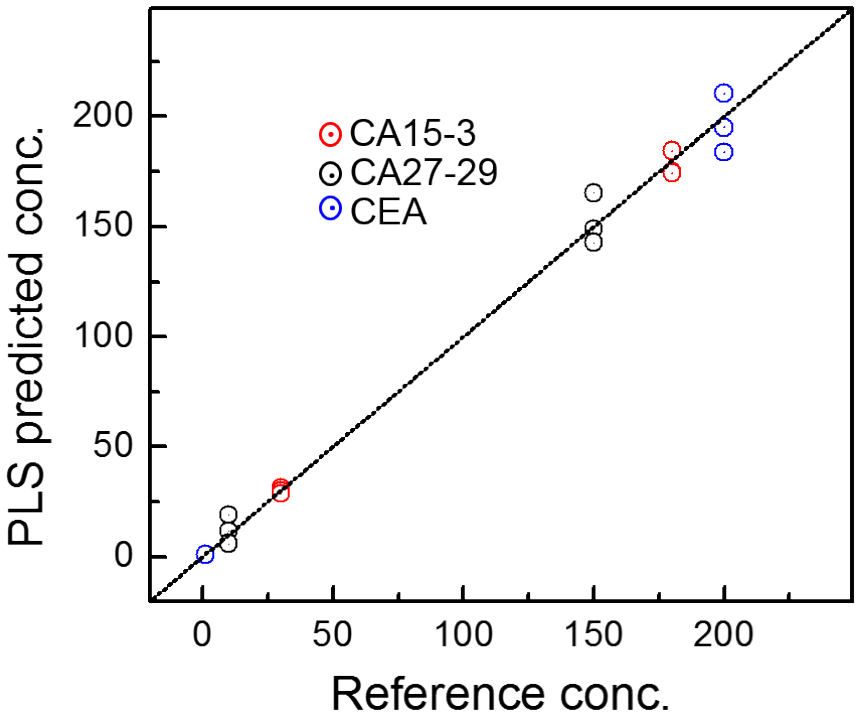


Figure S6. PLS regression analysis results for CA15-3, CA27-29 and CEA. The solid line denotes $y=x$ values. Samples were prepared by spiking the biomarkers in FBS (0.1, 1.0, 10, 50, 100 and 500 U/mL for CA15-3 and CA27-29, and 0.1, 1.0, 10, 50, 100 and 500 ng/mL for CEA).



Biomarker	REP	RSD	R^2
CA15-3	10.4%	13.5%	0.98
CA27-29	3.0%	4.0%	0.99
CEA	6.0%	8.4%	0.99

Figure S7. PLS regression analysis of serum samples with healthy concentrations and patient biomarker concentrations. Table lists the resultant parameters from the PLS regression.