Supporting information

Effect of different-sized spherical gold nanoparticles grown layer by layer on the sensitivity of immunochromatographic assay

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1. Optimization of anti-OTA ascitic fluid-saturated labeled concentrations with different-sized AuNSs.

Anti-OTA ascitic fluids were used to label four sizes of AuNSs directly. The pH of AuNS solutions were adjusted by adding K_2CO_3 (0.2 mol/L) to a final pH of 6.5 for labeling anti-OTA ascitic fluids. Anti-OTA ascitic fluid-labeled, different-sized AuNSs were optimized by dropping a series of concentrations of anti-OTA ascitic fluids to 1 mL of pH adjusted AuNS solutions. The saturated labeled amounts of anti-OTA ascitic fluids on different-sized AuNSs were evaluated by recording the OD_T value after running the same amount of AuNS probes on strips. Fig. S1 indicates that the OD_T value of the strip increases sharply with increasing ascitic fluid content and then gradually declines with further increase in ascitic fluids. We speculate that an excessive density of mAbs on the surface of AuNSs would lead to steric hindrance of the mAbs and, in consequence, bad color on the test line. Thus, the saturated labeled amounts of anti-OTA ascitic fluids were 14.21 μg per mL of 20 nm conventional AuNS solution on AuNSs (1.0 nmol/L, purple line). The saturated labeled amounts of anti-OTA ascitic fluids for 60 (blue line), 100 (red line), and 180 nm (green line) AuNS solution (10 pmol/L) were 5.62, 11.84, and 25.64 μg, respectively.

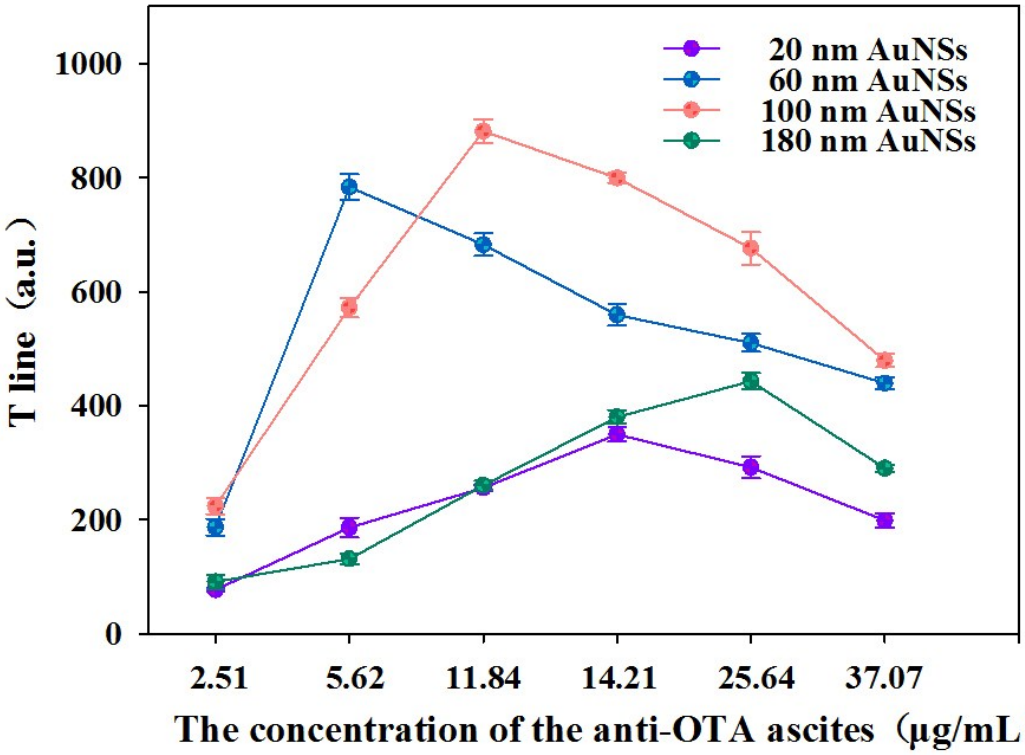

Figure S1. Preparation and characterization of AuNS probes. Optimized saturated labeled amounts of anti-OTA ascitic fluids on AuNSs.

2. Kinetic screening of different-sized AuNS probes and OTA–BSA artificial antigen

Binding curves for the reported K_D values for different-sized AuNS probes and OTA–BSA artificial antigen were obtained from bio-layer interferometry experiments as previously described. K_D values are summarized in Table 1 in the main body of the manuscript.

A. 20 nm AuNS probes, $K_D = 6.004\times10^{-11}$ M

B. 60 nm AuNS probes, $K_D = 4.721\times10^{-11}$ M

C. 100 nm AuNS probes, $K_D = 4.424\times10^{-11}$ M

D. 180 nm AuNS probes, $K_D = 6.435\times10^{-12}$ M

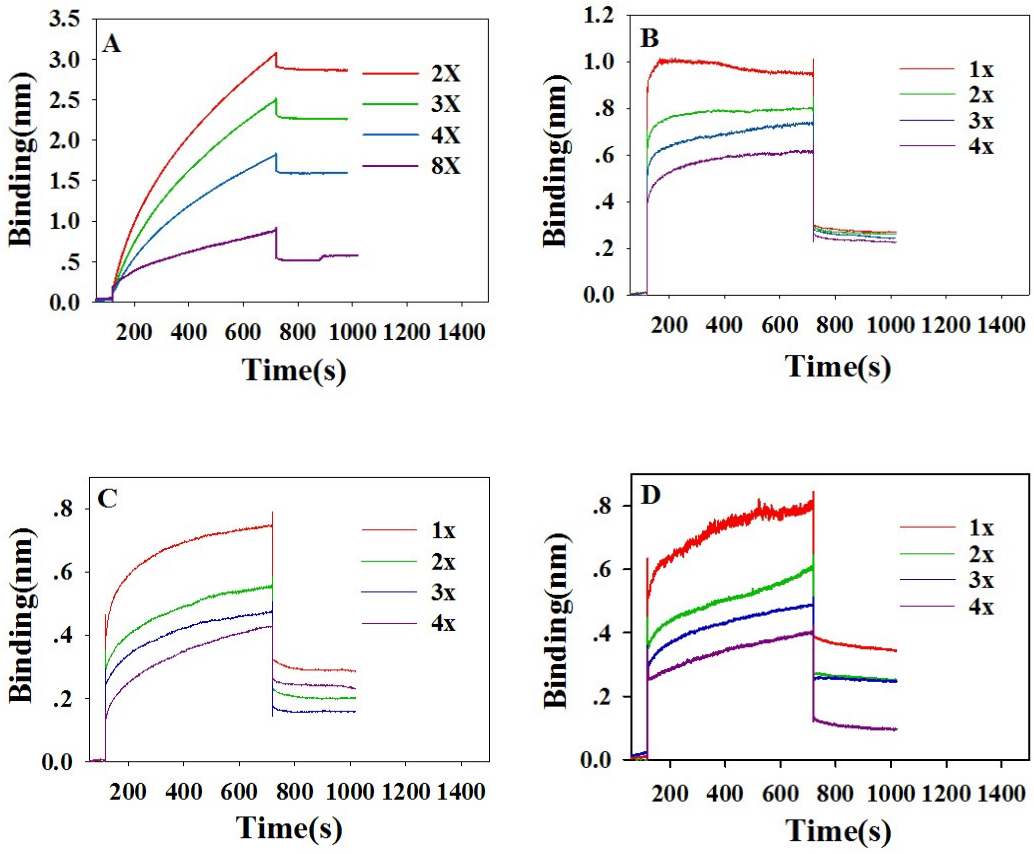

Figure S2. Kinetic screening of different-sized AuNS probes and OTA–BSA artificial

antigen using ForteBio Streptavidin (SA) biosensors. A. 20 nm; B. 60 nm; C. 100 nm; D. 180 nm. In addition, "*Nx*" and "*nx*" represent the dilution ratio of AuNS probes. 2*X* = 1.174 nmol/L, 3*X* = 0.587 nmol/L, 4*X* = 0.391 nmol/L, 8*X* = 0.294 nmol/L; 1*x* = 11.74 pmol/L, 2*x* = 5.87 pmol/L, 3*x* = 3.91 pmol/L, 4*x* = 2.94 pmol/L.

3. Comparison of color intensity of different-sized AuNS-based ICA strip

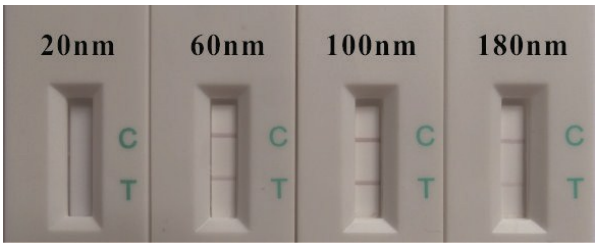

Figure S3. Stereogram of strips with 0.235 fmol of different-sized AuNS probes in each strip.

4. Cut-off values of different-sized AuNS-based ICAS (wet method)

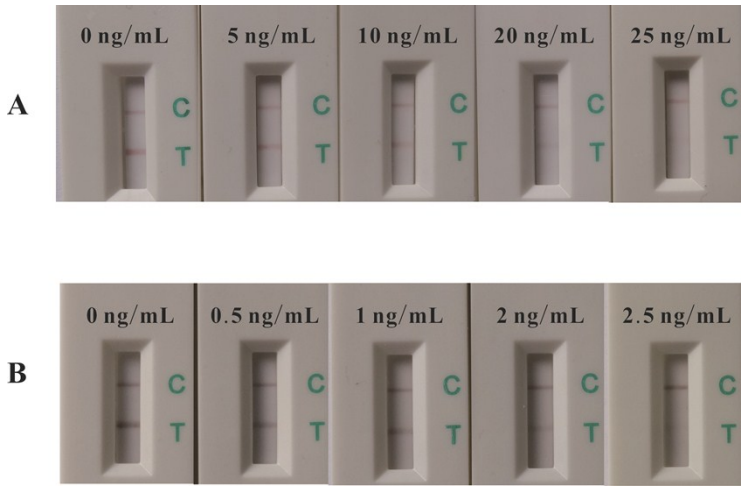

Figure S4. Physical pictures of two-sized AuNS (**A.** Conventional 20 nm AuNSs; **B.** 100 nm AuNSs) based ICAs for the detection of different OTA concentrations.

64 **Table S1.** Optimization of the parameters of the 20 nm AuNS-based strip[#].

No.	Concentration of OTA-BSA (mg/mL)	Amount of AuNSs-probe in each strip (fmol)	Optical density of T-line (a.u)	Optical density of C-line (a.u)	Value of T/C	Inhibition rate (%)
1	1.99	7.05	824.1 ±10.2	525.7 ± 3.93	1.567 ± 0.07	7.40 ± 1.11
2	1.59	7.05	686.3 ±3.81	528.7 ± 4.57	1.298 ± 0.15	3.62 ± 0.17
3	1.32	7.05	580.5 ±2.91	535.0 ± 9.11	1.085 ± 0.27	10.23 ± 2.21
4	1.99	4.7	593.2 ±6.46	330.6 ± 11.0	1.794 ± 0.14	30.22 ± 1.93
5	1.59	4.7	450.2 ±6.95	336.2 ± 2.91	1.339 ± 0.10	29.06 ± 1.21
6*	**1.32**	**4.7**	**416.1** ±3.30	**353.9** ± 4.80	**1.176** ± 0.05	**44.29** ± 0.80
7	1.99	2.35	486.4 ± 11.3	262.9 ± 3.91	1.850 ± 0.08	36.81 ± 4.81
8	1.59	2.35	344.7 ± 9.11	276.0 ± 7.25	1.249 ± 0.18	18.65 ± 3.39
9	1.32	2.35	313.6 ± 2.25	252.0 ± 9.02	1.244 ± 0.11	40.09 ± 4.16

65 Notice: * The optimal combinations.

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