Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Lateral Flow Assays for Ochratoxin A Using Metal Nanoparticles: Comparison of "Adsorption-Desorption" approach to Linkage Inversion Assembled Nano-Aptasensors (LIANA).

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Table of Contents:

I. Synthetic Procedures

a) Materials and Methods

Gold (III) chloride hydrate ($HAuCl_4.3H_2O$, ≤ 99.9%), Silver nitrate ($AgNO_3$, ≤ 99.9%), Sodium citrate, Sodium chloride, Sodium borohydride ($NaBH_4$, 99%), N-2-hydroxyethylpiperazine- N'-2'-ethanesulfonic acid (HEPES), and Streptavidin were purchased from Sigma-Aldrich. Ochratoxin A (OTA) and warfarin standards were purchased from Sigma-Aldrich. Ochratoxin B (OTB) was purchased from Santa Cruz Biotechnology Canada. A08min-aptamer was purchased from Integrated DNA Technologies (IDT), the aptamer contained a biotin moiety at one end and thiol moiety is other end. Linker DNA (3'-AGCAC-5'-5'-CACGA-3') was purchased from University Core DNA Services, University of Calgary, Canada. All buffers were prepared with Millipore Milli-Q deionized water at 18MΩ. TEM images were recorded using FEI Tecnai F20 FETEM in Carleton University; Chemistry Dept. UV/Vis absorption spectra were obtained using a CARY 300 Bio spectrophotometer (Varian, USA). A high-speed Sorvall legend micro 21R (Thermo electron corporation) centrifuge was used for the centrifugation of the solution.

b) Buffer

A08min	Buffer	10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, and 137 mM NaCl	pH 7.4

c) Aptamers and linker DNA

A08min aptamer (40 base pair)	5'-HS-GGC AGT G TG GGC GAA TCT ATG CGT ACC GTT CGA TAT CGT G-3-BioT' 3'-AGCAC-5'-5'-CACGA-3' (linker DNA)

d) Gold nanoparticle (AuNP) synthesis

Procedures for colloidal gold nanoparticle preparation were essentially the same as those developed by Grabar *et al*, [1] except with a decrease in the ratio of $HAuCl_4$ to sodium citrate, since higher $HAuCl_4$ concentration leads to larger colloids. [2] All glassware used for AuNP synthesis was cleaned by

soaking in aqua regia (3:1 mixture of concentrated HCl/HNO_3) for 15 minutes followed by thorough rinsing with deionized water. A 250 mL Erlenmeyer flask was used to mix 98 mL of deionized water and 2 mL of 50 mM $HAuCl_4$ to a final concentration of 1 mM $HAuCl_4$. The solution was heated to boiling with stirring. Once boiling, 10 mL of 38.8 mM sodium citrate was added. Following the change in suspension color from pale yellow to dark blue and finally to a wine red, heating was continued for an additional 20 minutes. The flask was removed from the heat and allowed to cool to room temperature with continued stirring. The nanoparticles were quantified by UV-Vis.

e) Synthesis of AgNPs

The citrate-stabilized AgNPs used in this study were synthesized following a procedure reported in the literature. [3] Briefly, 250 µL of 100 mM $AgNO_3$ and 250 µL of 100 mM trisodium citrate were added into 100 mL of water under stirring. Then 6 mL of freshly prepared 5 mM $NaBH_4$ was added into the above aqueous solution under vigorous stirring. The resulting yellow colloidal silver solution was further stirred for 30 min and then was left undisturbed overnight. Finally, the AgNPs were purified by centrifugation at 14 000 rpm for 30 minutes to remove the excess citrate molecules in the supernatant solution. The AgNPs had a plasmon absorption peak around 390 nm.

k) Assembly of a lateral flow assay strips

The Millipore Hi-FlowTM Plus Assembly Kit (Millipore Corporation, Bedford, MA) was used. The kit contains a Hi-Flow Plus Cellulose Ester Membrane (HF18004XSS HiFlow Plus 180 4mil 30.5 cm x 25 cm) and placed on an adhesive card. The length of the membrane along the flow direction is 2.5 cm on the backing. The absorption pad and wicking pad were cut from Millipore cellulose fiber sample pads (CFSP223000 SureWick CFSP 20 cm x 30 cm, Millipore) and the conjugation pad was cut from the Millipore glass fiber conjugate pad (GFDX203000 SureWick GFDX 20 cm x 30 cm, Millipore). The absorption pad, wicking pad, and conjugation pad were attached to the adhesive card of the membrane in a way as shown in Figure S1A. The overlap for each pad was approximately ~ 2 mm and the width was ~5 mm, cut by a paper cutter.

For the LIANA method (Figure S1B and C), only a test dot (streptavidin, 2.5 mg/ml) is located on the nitrocellulose membrane. The nitrocellulose membrane has a high affinity for streptavidin due to its electrostatic charge. The test dot only changes color when the OTA (target) is present in the sample, otherwise no color should be present.

For the adsorption-desorption method (Figure S2), the test and control dots (streptavidin 2.5 mg/ml, 20% PDDA polymer, 1µL) were located on the nitrocellulose membrane. The test dot is not observed when the OTA (target) is present in the sample, in the absence of OTA the color should be present.

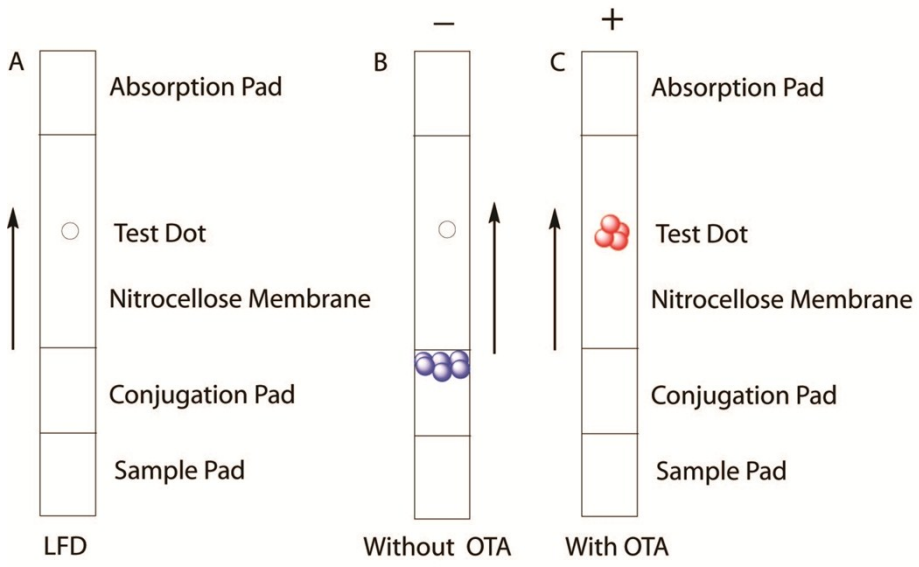

Figure S1. Schematic illustration of LIANA lateral flow assay A). Assembly of a lateral flow Device (LFA). B) Absence of OTA, the nanocomplex **1** (the aggregates, on the conjugate pad) was stop flows, No color was observed on the test dot. C) Presence of OTA, OTA-induced disassembly of nanocomplex **1**, the dispersed nanoparticles are captured by streptavidin, and red color was observed on the test dot.

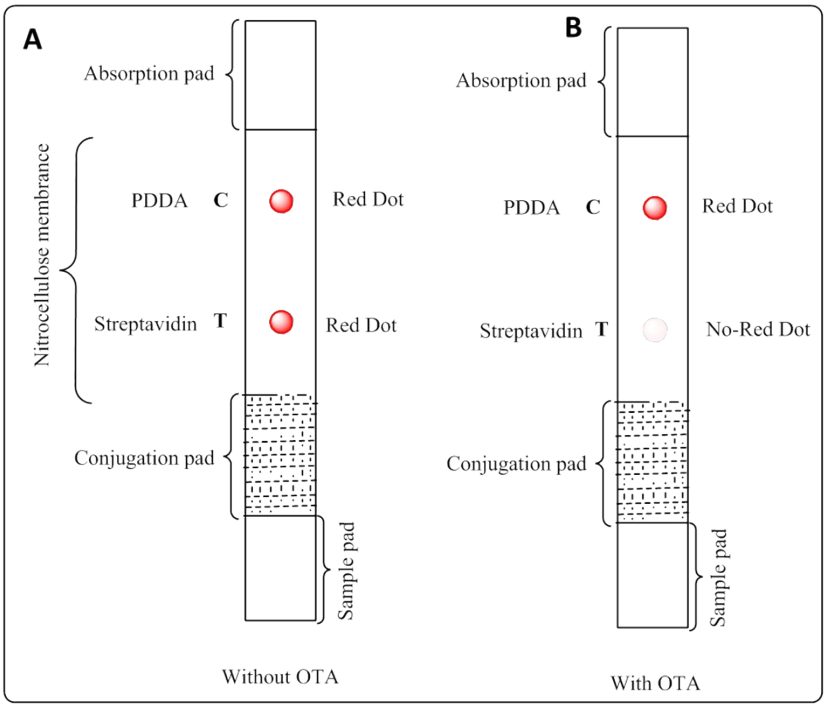

Figure S2. Schematic illustration of the Adsorption-Desorption colorimetric lateral flow assay. The test and control dots were (streptavidin, 2.5 mg/ml, 20% PDDA polymer, 1 µL were located on the nitrocellulose membrane. A) Absence of OTA, color was observed on the test dot. In the presence of OTA, no red color dot was observed on the test dot.

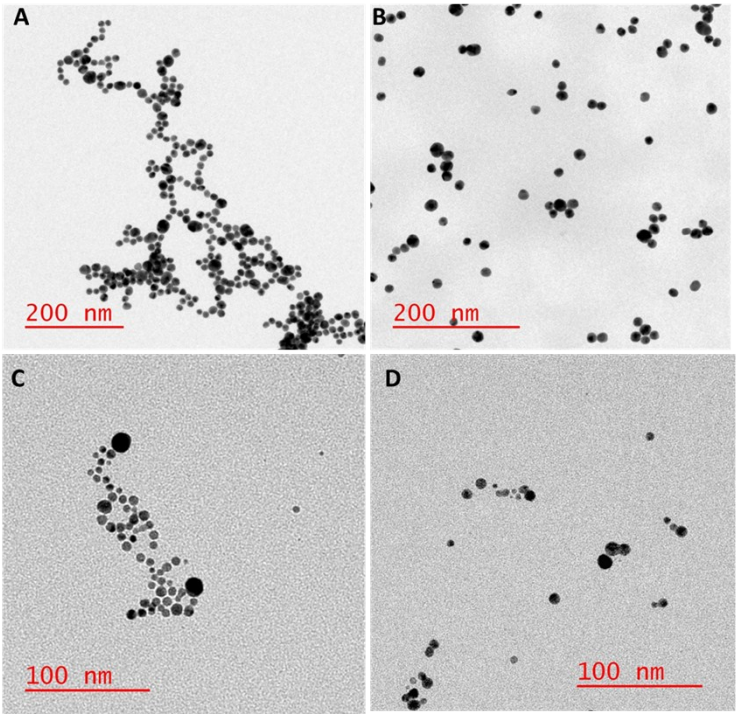

Figure S3. HR TEM images of (A) A08min-AuNPs in the presence of (0.1 µM) a 5'-5' linker DNA (formation of assembly) and (B) after 30 minutes incubation with 10 µM OTA (disassembly of of nanocomplex **1**). Conditions: Concentrations of OTA [10 µM], AuNPs [460 pM], Linker DNA [0.1 µM] and A08min [0.1 µM]; Buffer. Similar observations were observed assembly and disassembly of A08min-AgNPs. (C) The A08min-AgNPs in the presence of a 5'-5' linker DNA and (D) addition of OTA. Conditions: Concentrations of OTA [10 µM], AgNPs [460 pM], 5'-5'Linker DNA [0.1 µM] and A08min [0.1 µM]; Buffer.

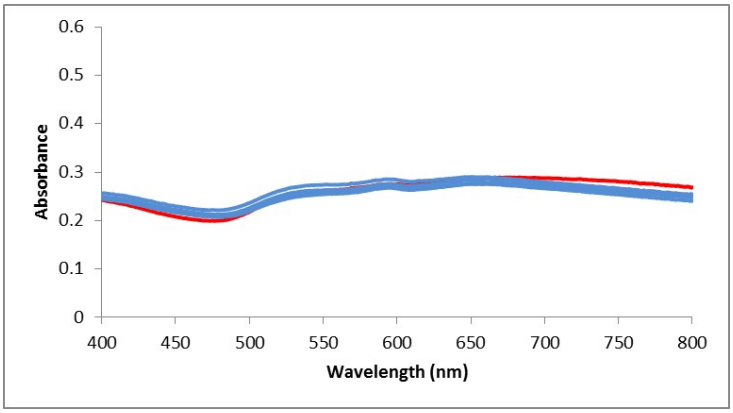

Figure S4.The absorption spectra of nanocomplex **2** with increasing concentrations of OTB. The absorption spectra of nanocomplex 2, upon the addition of 0–63 μM of OTB. The intensity (absorbance) is no significant change (top red line is nanocomplex **1**). All experiments were performed in buffer solution (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, and 137 mM NaCl, pH 7.4).

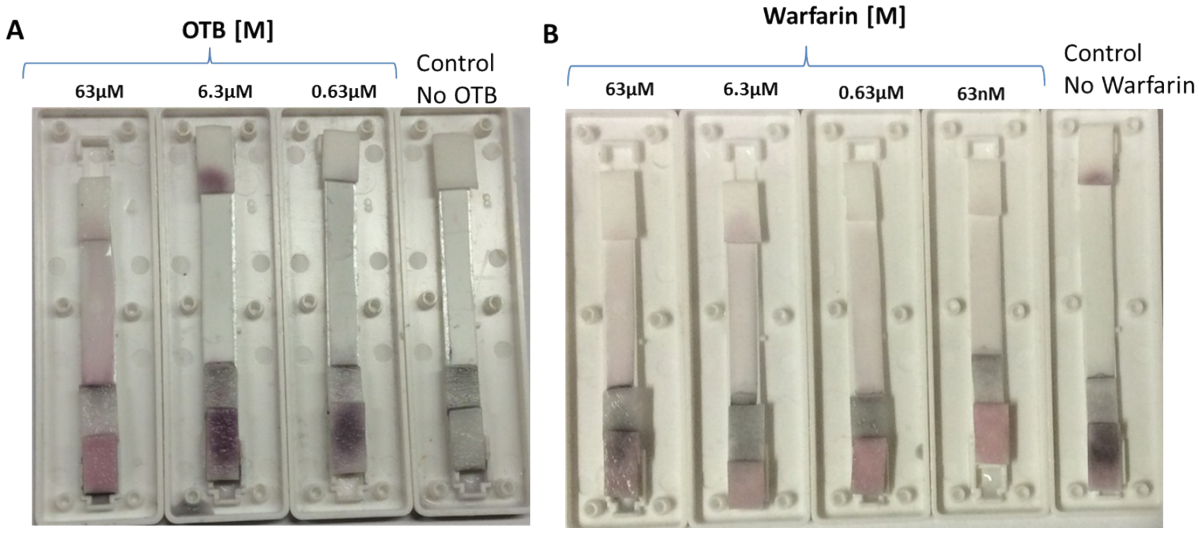

Figure S5. A) The LIANA lateral flow assays (LFA) for the mixture of A08min-AuNPs and a 5'-5' linker DNA with different concentrations of OTB (0.63, 6.3, and 63 µM) and absence of OTB. The LFA test dot is no significant color change. Similar observations were observed with different concentrations of warfarin (63nM, 0.63, 6.3, and 63 µM) and absence of warfarin. The LFA test dot is no significant color change.

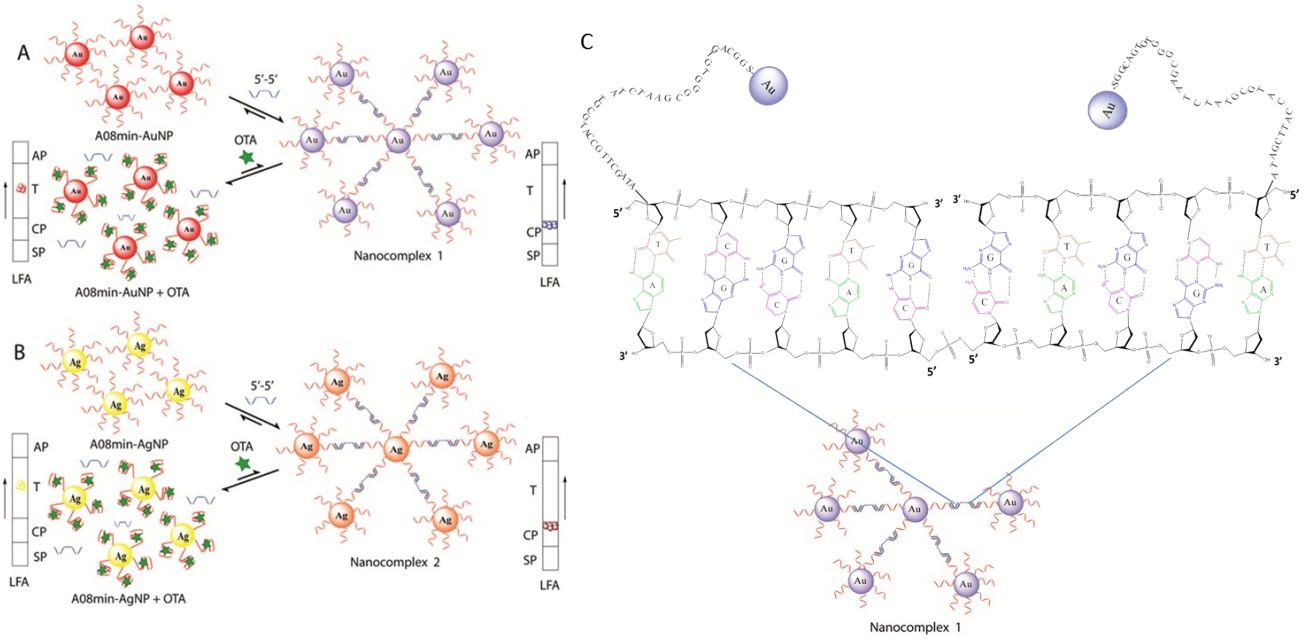

Figure S6. Schematic illustration of colorimetric lateral flow assays for the detection of OTA based on Linkage Inversion Assembled Nano-Aptasensors (LIANAs). A) AuNPs (Red spheres) functionalized with OTA aptamers (A08min) are aggregated (nanocomplex 1, Purple spheres) by a 5'-5' linker DNA and the nanocomplex 1 surface plasmon (SP) is shifted towards longer wavelength. In the presence of OTA, OTA binds with its aptamer and dissociation of nanostructure 1 (Red spheres). B) Is aggregated in a similar fashion, with silver nanoparticles (brown spherical) and A08min were functionalized AgNPs in the presence of a 5'–5' linker DNA and the formation of assembled nanocomplex 2. OTA-induced disassembly of the nanocomplex 2 (yellow sphericals). C) Schematic illustration of the 5'-5' linkers were hybridized with the apatamer.

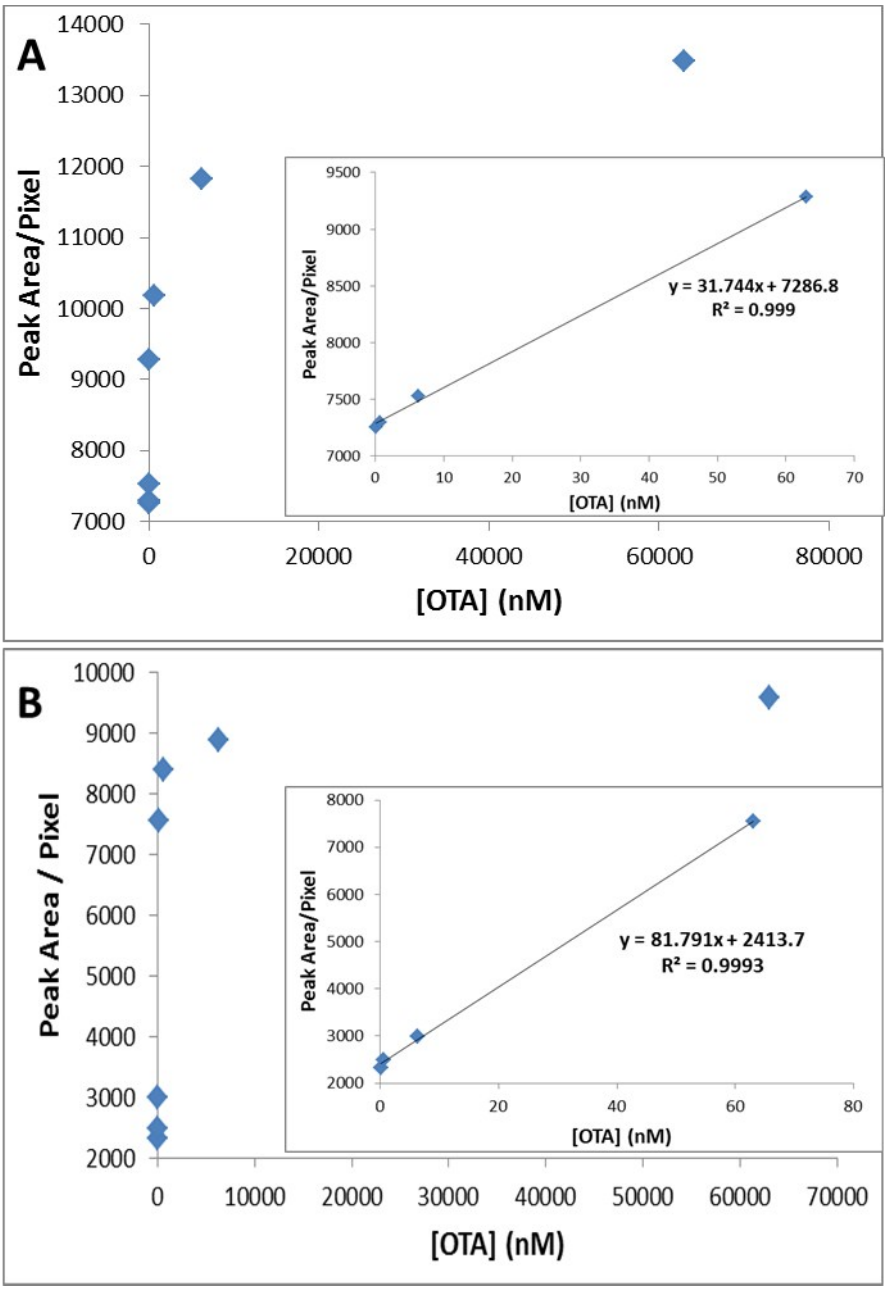

Figure S7. Calibration curve of OTA, LIANA based LFA sensing system: the curve was plotted as the peak area of test zone (T) vs various OTA concentrations. Inset shows a linear relationship between T and the OTA concentration (0.06 to 63 nM). A) AuNPs B) AgNPs

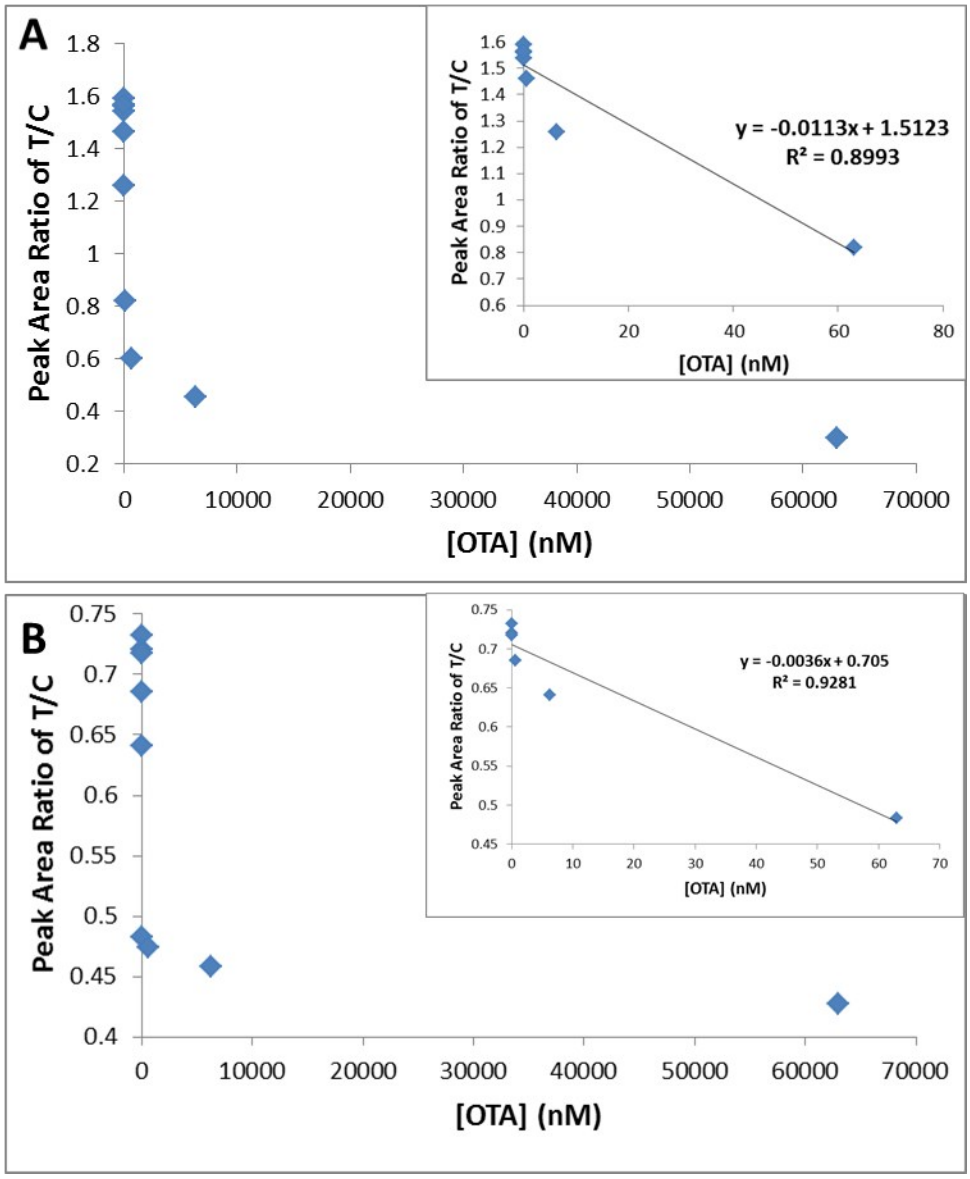

Figure S8. Calibration curve of OTA adsorption-desorption LFA sensing system: the curve was plotted as the peak area ratio of test and control zones (T/C) vs different OTA concentrations. Inset shows a linear relationship between T/C and the OTA concentration (0.06 to 63 nM). A) AuNPs B) AgNPs

III. References

1 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem,* 1995, ***67*****,** 735-43

2 G. Frens, *Nat. Phys. Sci.* 1973, **241**, 20-22

3 R. C. Doty, T. R. Tshikhudo, M. Brust, and D. G. Fernig, *Chem. Mater*. 2005, **17**, 4630–4635.