Supporting Information for

Luminous Silica Colloids with Carbon Dots Incorporation for Sensitive Immunochromatographic Assay of Zika Virus

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Materials

N-β-(aminoethyl)-γ-aminopropyltrimethoxysilane (AEAPTMS), Citric acid, cetyltrimethylammonium bromide (CTAB), sodium salicylate, TEOS, triethanolamine (TEA), succinic anhydride, N, N dimethylformamide (DMF), Hydrochloric acid (HCl), ethanol, ammonia aqueous solution (28%), Sodium chloride (NaCl), Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, ≥99.0%), poly(vinyl pyrrolidone) (PVP, Mw=40K) were purchased from were purchased from Sinopharm Chemical Reagent Co., Ltd. Potassium carbonate (K_2CO_3, ≥99.0%) and sucrose ($C_{12}H_{22}O_{11}$) were obtained Nanjing chemical reagents Co., Ltd. (Nanjing, China). Tween-20 was supplied by Nanjing Ding Biology Technology Co., Ltd. Bovine serum albumin (BSA) was obtained from Sangon Biotech Co., Ltd. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was purchased from Energy Chemical Co., Ltd. Zika NS1 protein, labeled antibody and coated antibody against Zika named ZK01, ZK02, goat antimouse IgG antibody, sample pads, nitrocellulose (NC) membranes, absorbent pads, and black plastic adhesive cards were purchased from Shanghai JieYi Biotechnology Co. Ltd. (Shanghai, China). Serums of patients infected with Zika virus and normal human serum were provided by Jiangsu Province Hospital. Data of serum detection using the PCR method were supplied by Jiangsu Province Hospital.

Characterizations

Transmission electron microscopy (TEM) measurements were carried out under a Tecnai G^2 20 transmission electron microscope (FEI.USA). The fluorescent spectra were performed on a FluoroMax-4 spectrofluorometer (Horiba, USA). Zeta potential was acquired with a Marlvern Zetasizer Instrument (Nano ZS, England). The UV-vis absorption spectra were recorded by a Shimadzu UV-2450 spectrophotometer (Japan). The fluorescent image of the FCS spheres was obtained using the FV3000 Olympus laser scanning microscope (Japan).

Synthesis of Silanized CDs

15 mL of AEAPTMS was poured into a 50 mL three-necked flask and purged with nitrogen for 15 min. The solution was then heated to 240°C, and 1 g of dehydrated citric acid (pretreatment of citric acid monohydrate in an 80°C vacuum oven overnight) was quickly added with vigorous stirring. After 5 min, the solution was stopped from heating and allowed to cool naturally. The solution was centrifuged at 10,000 rpm for 10 min, and the large particles at the bottom were removed to obtain CDs.

Synthesis of Dendritic SiO_2 Spheres

68 mg of TEA was added into 25 mL of H_2O and stirred at 80°C for 30 min, then 380 mg of CTAB and 168 mg of sodium salicylate was added and stirred for 1 h. Injected 4 mL of TEOS into the solution and stirred gently at 80°C for 2 h. The mixture was diluted with ethanol and collected by centrifugation. The precipitates were washed with ethanol for several times and finally dispersed in HCl/ethanol mixture and stirred at 60°C for 24 h to extract the residue organic templates. The dendritic SiO_2 spheres were washed with ethanol and H_2O and finally dispersed in ethanol.

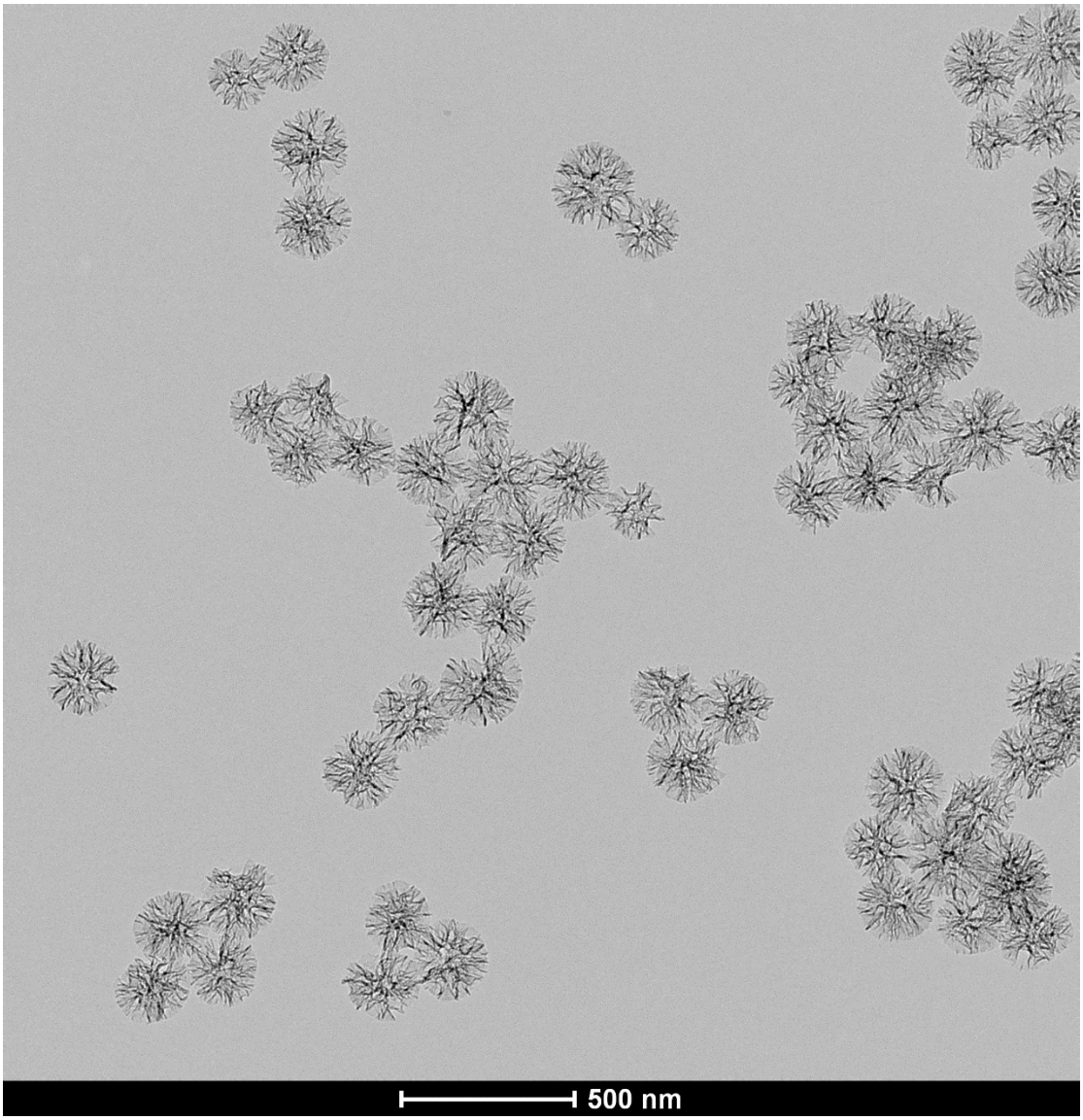

Figure S1. TEM image of dendritic SiO_2 spheres.

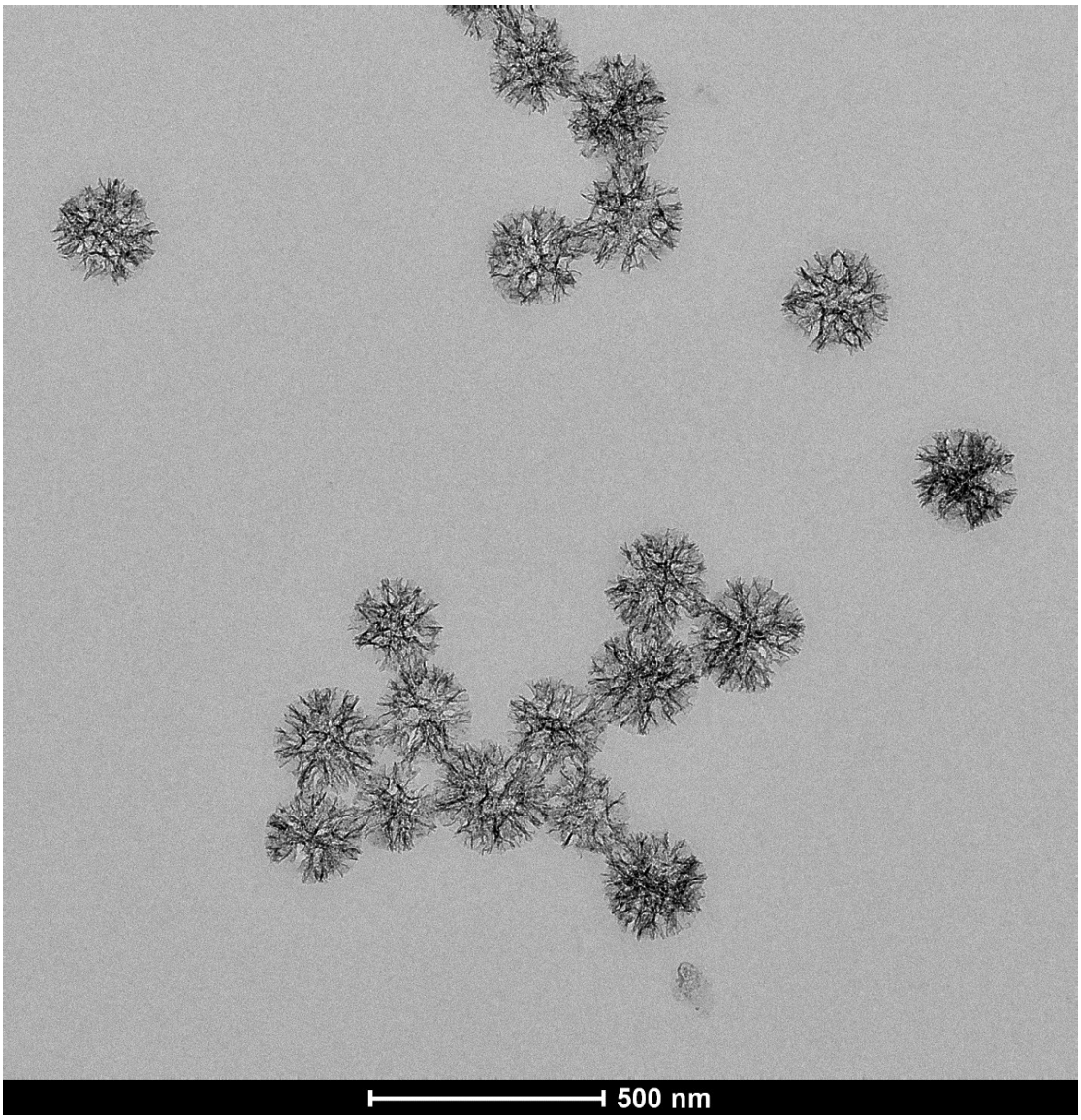

Figure S2. TEM of CDs incorporated silica spheres.

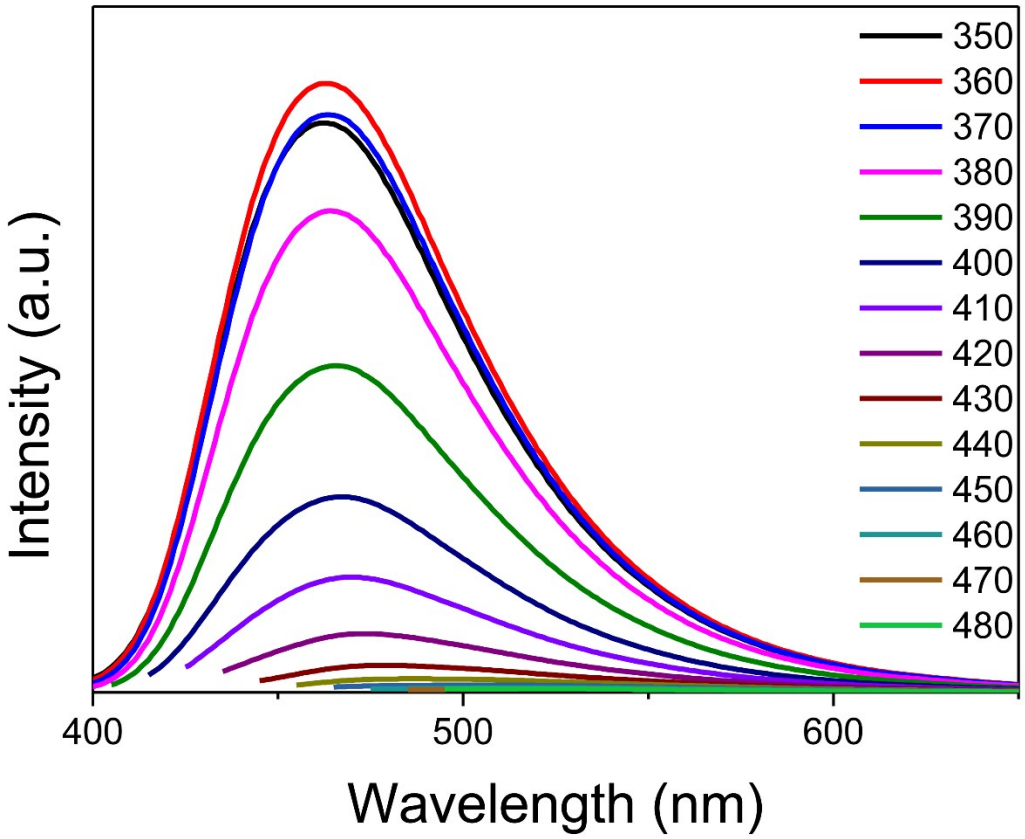

Figure S3. Emission spectra of CDs aqueous solution at different excitation wavenumbers.

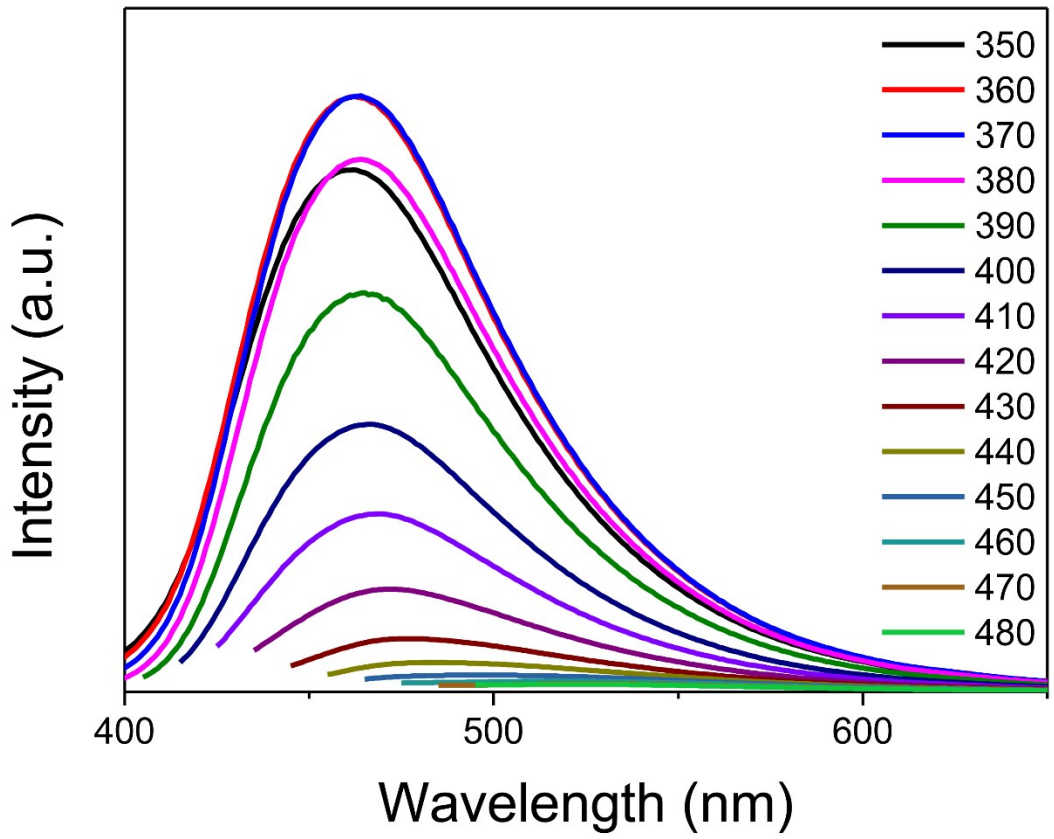

Figure S4. Emission spectra of FCS spheres aqueous solution at different excitation wavenumbers.

Theoretical Calculation of the Composition of CDs

According to the molecular weight (222) and density (1.010 g/mL (25 °C)) of AEAPTMS, the molar content of 15 mL AEPTMS is 6.82×10^{-2} mol. According to the molecular weight of dehydrated citric acid is 192.13, the molar content of 1g dehydrated citric acid is 5.20×10^{-3} mol. In addition, the molar ratio of citric acid to AEAPTMS reaction is 1: 3, because one citric acid molecule contains three carboxyl groups, and one AEAPTMS molecule contains one primary ammonia that reacts with carboxyl groups. Even under ideal conditions, citric acid is completely reacted, and the consumption of AEAPTMS is at most 1.56×10^{-2} mol. That is, the consumption of AEAPTMS accounts for 22.87% of the total AEAPTMS. In brief, the silanized CDs contain a large amount of AEAPTMS. In the subsequent process of silanized CDs embedded in dendritic silica, AEAPTMS also participated in this process, so FCS spheres are rich in amino groups.

Synthesis of Au NPs

The preparation of Au NPs was carried out according to the reported methods.[S1] 5 mL of 0.2% chloroauric acid aqueous solution was added to 95 mL of H_2O, placed in a round-bottom flask and heated to 100°C to boil. Upon boiling, 1 mL of freshly prepared 1% trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) aqueous solution was quickly added under vigorous stirring. When the color of the solution becomes completely transparent wine red, continue boiling for 15 min. Then continue stirring at room temperature to cool to room temperature, and make up to 100 mL after cooling. Stored at 4°C and keep away from light.

Preparation of Au NPs-ZK01 Antibody Conjugates

Au NPs-ZK01 antibody conjugates were prepared according to the method previously reported with appropriate modifications.[S2,S3] In brief, a certain volume of potassium carbonate aqueous solution (K_2CO_3, 0.1 M) was added to 200 μL of Au NPs solution to make it pH 8, and then 5 μL of 1 mg/mL ZK01 antibody was added to the solution and reacted for 30 min. Thereafter, 25 μL of 5% BSA was added and shaken for 30 min. The solution was centrifuged at 1500 rpm for 10 min to remove the bottom precipitate. The supernatant was further centrifuged at 15000 rpm for 30 min and the supernatant was removed. The obtained product was redispersed in 100 μL PBS (0.01 M, PH = 7.4, 5% Sucrose, 1% BSA) and stored at 4°C.

Fabrication of the Au NPs-Based Lateral Flow Test Strip

The sample pads were treated with 10 mM PBS (pH = 7.4) containing 0.1% (v/v) Tween-20 and 0.5% PVP and dried at 37°C. The conjugate pads were treated with 10 mM PBS (pH = 7.4) containing 0.1% (w/v) Tween 20, 4% (w/v) sucrose and 2% (w/v) BSA and dried at 37 °C. The ZK02 antibody (2 mg/mL) and the goat antimouse IgG antibody (2 mg/mL) in PBS was dispensed at the test and the control line on the nitrocellulose membrane, respectively, using a double headed marker (SJ001, Shenzhen Stationery Store) with an extremely small nib (SJ002, 1.97 × 34 mm, Shenzhen, Stationery Store) and drying at 37 °C for 2 h. The Au NPs-ZK01 antibody conjugates dispersion was applied to the treated conjugate pad and then dried at 37°C for 40 min. The absorbent pad, nitrocellulose membrane, pretreated conjugate pad, and pretreated sample pad were attached to a black plastic backing and assembled as a strip, followed by cutting into 3 mm wide pieces. The obtained strips were stored at 4°C until use.

Detection of Zika NS1 Protein Using Au NPs-Based Lateral Flow Assay

The Zika NS1 protein standard solution (60 μL) was added onto the sample pad of the Au NPs-based lateral flow test strip, and the solution was transferred to the absorbent pad under the capillary force. The presence of target proteins was judged by the specific color tracer of Au NPs. After 20 min, the results were observed with the naked eye.

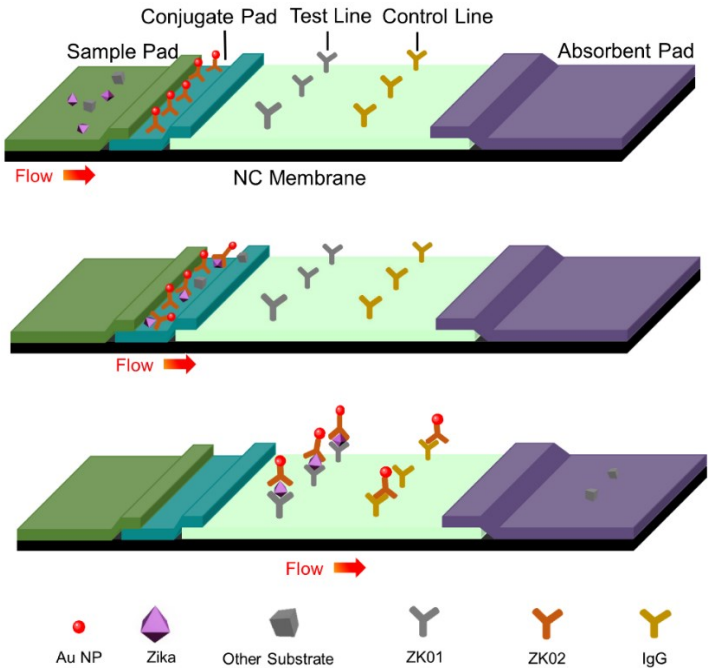

Figure S5. Schematic representation for the Zika NS1 protein detection using Au NPs-based lateral flow immunoassay.

To perform the Au NPs-based lateral flow immunoassay, the liquid sample containing Zika NS1 protein was dropped onto the sample pad and migrated along with the strip driven by capillary forces. The Au NPs-ZK01 antibody conjugates preadsorbed on conjugate pad was then rehydrated to form immunocomplex with Zika NS1 protein. When transported to the test line, the immunocomplex was captured by preimmobilized ZK02 antibody to form Au NPs-ZK01/NS1/ZK02 sandwich structure, via specific antigen-antibody interaction. Whereas excess Au NPs-ZK01 antibody conjugates further flowed to the control line and trapped by preimmobilized goat antimouse IgG to form Au NPs-ZK01/IgG structure, which determines the validity of individual test. After 20 min of the assay, the results were read directly by visual.

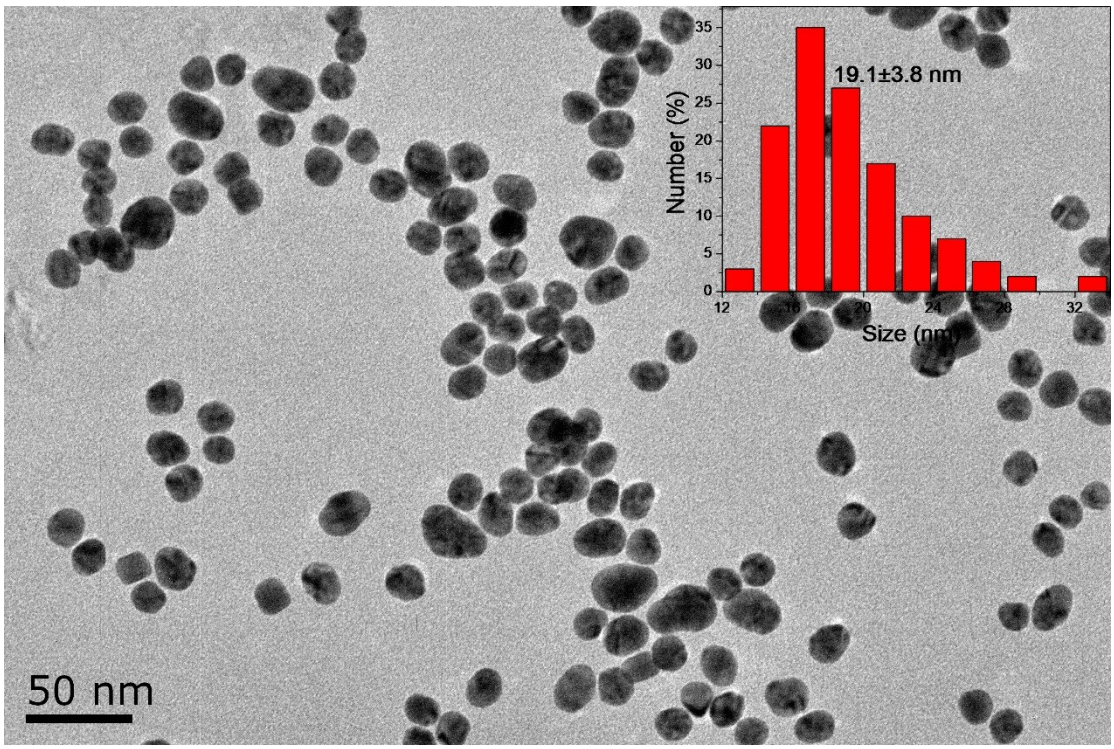

Figure S6. TEM image of Au NPs.

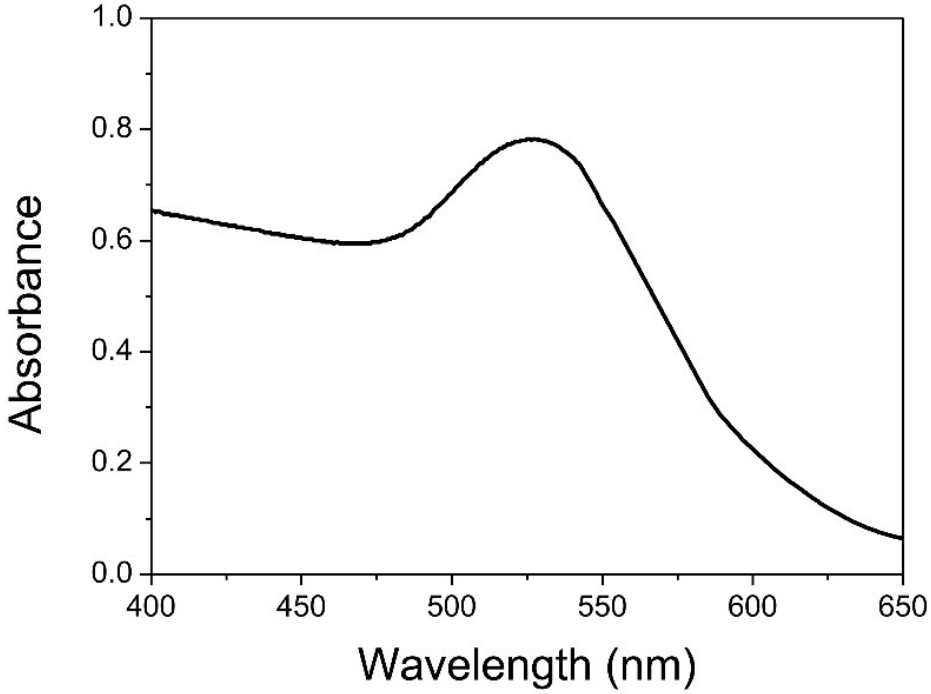

Figure S7. UV-vis spectra of Au NPs.

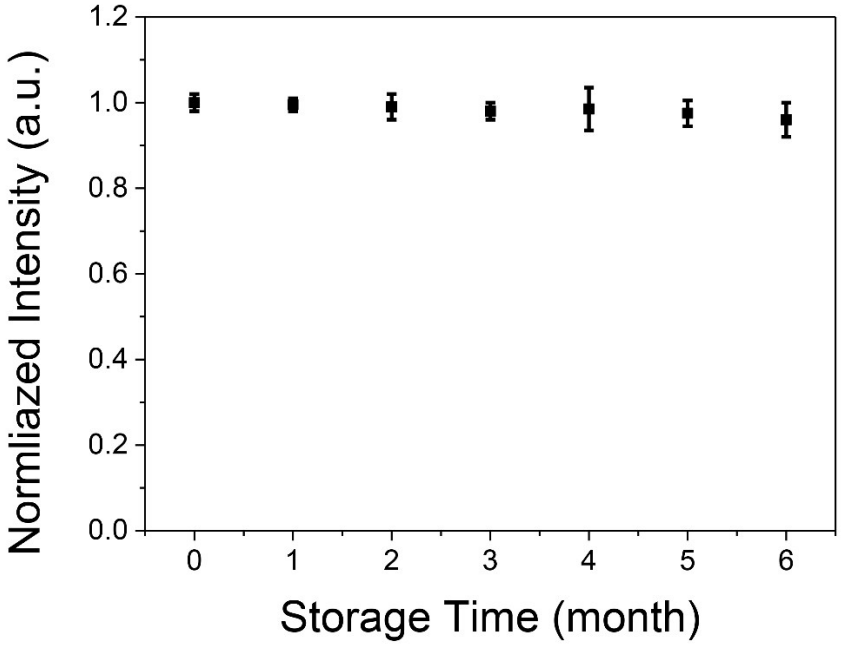

Figure S8. Stability test of the iFCS at different storage times.

Details of Interferents

HCG (human chorionic gonadotropin) is a glycoprotein hormone secreted by syncytiotrophoblast cells of placenta; AFP (Alpha-fetoprotein) is a glycoprotein, mainly used as a serum marker for primary liver cancer; CEA (carcinoembryonic antigen) is a broad-spectrum tumor marker; SFTSV (Severe fever with thrombocytopenia syndrome virus) can cause severe fever with thrombocytopenia syndrome, which is an acute infectious disease. STX2 (Shiga toxin type II) is a type of Shiga toxin. Shiga toxin is a potent toxin produced by Shigella type I dysentery. It has cytotoxicity, enterotoxicity and neurotoxicity. It is the main cause of serious diseases secondary to dysentery.

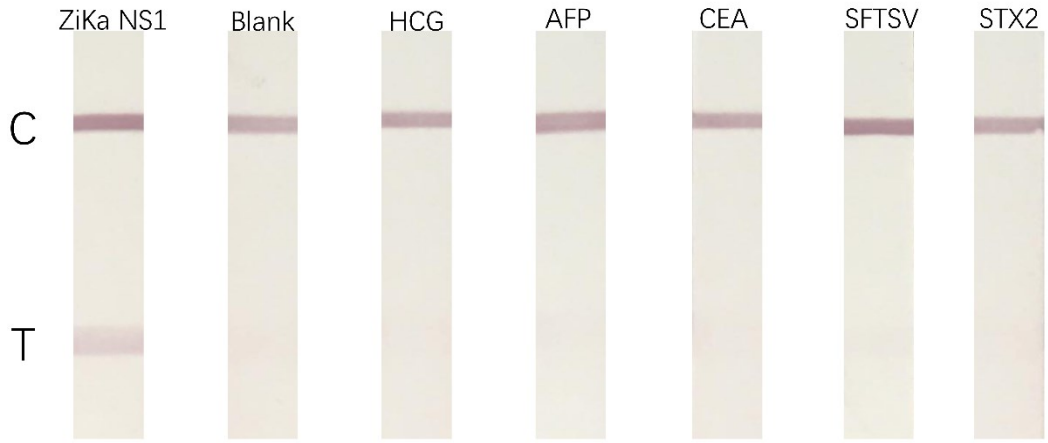

Figure S9. Specificity of Au NPs-based lateral flow test strips. From left to right: Zika NS1 protein, blank control, HCG antigen, AFP antigen, CEA antigen, SFTSV antigen and STX2 antigen. All concentrations were 1 μg/mL.

Table S1. Summary of analytical performances of Zika NS1 detection with different methods.

Method	Limit of detection	Assay time	Ref
Aptamer-Based ELISA Assay	0.1 ng/mL	12.5 min	S4
Novel graphene-based biosensor	22.5 ng/mL	20 min	S5
Surface-enhanced Raman spectroscopy immunoassay	10 ng/mL	2 h	S6
Immunochromatography	18 ng/mL	15-60 min	S7
SERS-based lateral flow immunoassay	0.72 ng/mL	unspecified	S8
Quantum dot microspheres-based lateral flow immunoassay	0.045 ng/mL	20 min	S9
Au NPs-based lateral flow immunoassay	1 ng/mL	20 min	this work
Multiple Carbon Dots Embedded Silica Spheres-based lateral flow immunoassay	0.01 ng/mL	20 min	this work

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