SUPPLEMENTARY INFORMATION

Rational design to improve the detection sensitivity of the sandwich-type lateral flow immunoassays

Feiqian Wang,[a] Yinuo Sun,[a] Muzi Jin,[a] Baoxin Zhang,[b] Yiwen Cui,[a] Dexin Kong,[a] Dongmei Li,[c] Xiluan Yan[d] and Aiping Fan*[a]

[a] School of Pharmaceutical Science and Technology, Faculty of Medicine, Tianjin University, Tianjin 300072, China

[b] The Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot, Inner Mongolia 010050, China

[c] Tianjin Medical University Cancer Institute & Hospital, Tianjin 300060, China

[d] School of Pharmacy, Jiangxi Medical College, Nanchang University, Nanchang 330006, China

*Corresponding author: fanap@tju.edu.cn

Materials and chemicals

Human chorionic gonadotropin protein (*h*CG 303) and anti-human *h*CG monoclonal antibody pairs (anti-*h*CG 103 which targets the alpha subunit of hCG and anti-*h*CG 104 which targets the beta subunit of hCG) were purchased from Bioeast Biotech. Co., Ltd. (Hangzhou, China). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were purchased from HeavyBio Inc (Shenzhen, China). Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4 \bullet 4H_2O$), biotin N-hydroxysuccinimide easter (biotin-NHS), hydroquinone, and sucrose were purchased from Heowns Biochem Technology Co., Ltd. (Tianjin, China). Streptavidin (SA), bovine serum albumin (BSA), and sodium caseinate were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Triton X-100, Tween-20, and artificial urine were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). NP-40 was purchased from Yuanye Bio-Chem. Tech. Co., Ltd. (Shanghai, China). Polyethylene glycol 20,000 (PEG-20,000) was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. Tris(hydroxymethyl) aminomethane (Tris) was purchased from Tianjin Guangfu Fine Chemical Research Institute. Dibasic sodium phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), potassium carbonate (K_2CO_3), hydrochloric acid (HCl), and sodium chloride (NaCl) were purchased from Jiangtian Chemical Technology Co., Ltd. (Tianjin, China). The sample pad, conjugate pad, enhancement pad, absorbent pad (Cat. No. DB-7), MAX-line label, and polyvinyl chloride (PVC) back card were purchased from Shanghai Jiening Biotech Co., Ltd. (Shanghai, China). The sample pad, conjugate pad and enhancement pad are same product (Glass cellulose membrane, Cat. No. GL061). The NC membrane (Sartorius, CN95) was purchased from Sartorius Corporation (Germany).

Apparatus

The SpectraMax M2 multifunctional microplate reader (Molecular Devices Corporations, USA) was employed to obtain the UV-vis spectra of Au NPs. Transmission electron microscope (TEM) images of Au NPs were acquired with a JEM-2100 F TEM (JEOL) model. The Malvern Nano ZS (Zetasizer Nano ZS, UK)

was used to detect the size of different Au NPs. Gold probes and antibodies were respectively sprayed on the conjugate pad and NC membranes via the XYZ dispensing platform (HGS510, Hangzhou Autokun Technology Co., Ltd., China). The cutting machine (Goldbio GD300, Shanghai Kinbio Tech. Co., Ltd., China) was employed to cut the assembled back plates into test strips. Photographs of the detection results were recorded through the camera of the Oppo Reno 6.

Supplementary experiments

Procedure for the optimization of the pH of conjugation: The initial pH of the gold colloidal solution was pH 5.5, which was determined by precision pH test paper. The pH was adjusted to 6.0, 6.5, 7.0, 7.5, and 8.0 using 0.1 M K_2CO_3. Then, 1 mL of gold colloidal solution (27 pM) at each pH was transferred to a centrifugation tube and mixed with 30 μL of 1 mg mL^{-1} *h*CG detection antibody (anti-*h*CG 104), respectively. After incubating at room temperature for 30 minutes with shaking, 100 μL of 10% NaCl was added to the mixture. After standing at room temperature for 60 minutes. The absorption spectra of each mixture were measured to examine the stability of the Au NPs. The results shown in Fig. 10A indicated that the optimal pH for conjugation was pH 7.

Procedure for the optimization of the amount of detection antibody: The pH of the gold colloidal solution was adjusted to pH 7 with 0.1 M K_2CO_3. Then, different amounts (5, 10, 15, 20, 25, 30 μg) of hCG detection antibody (anti-*h*CG 104) were mixed with 1 mL of pH-adjusted gold colloidal solution (27 pM). The following procedures were similar to the above. The absorption spectra (Fig. 10B) showed that the minimum amount of anti-*h*CG 104 to stabilize 1 mL of Au NPs (27 pM) is 20 μg. The optimal amount of anti-*h*CG 104 for the preparation of the conjugates should be 20% more than the minimal amount. Therefore, 24 μg of anti-*h*CG 104 was employed for the preparation of the conjugates.

Supplementary figures

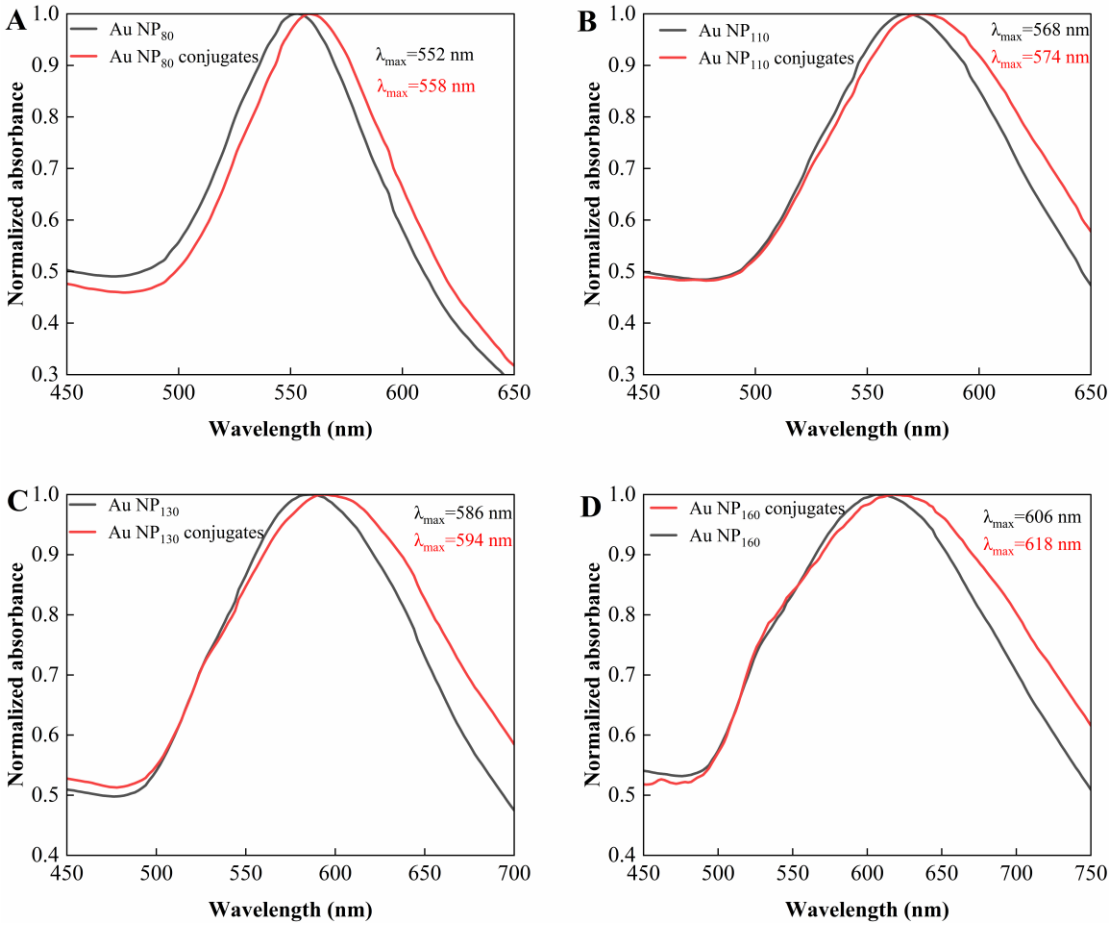

Fig. S1 The visible absorption spectra of the (A) Au NP_{80}, (B) Au NP_{110}, (C) Au NP_{130}, (D) Au NP_{160} and their conjugates.

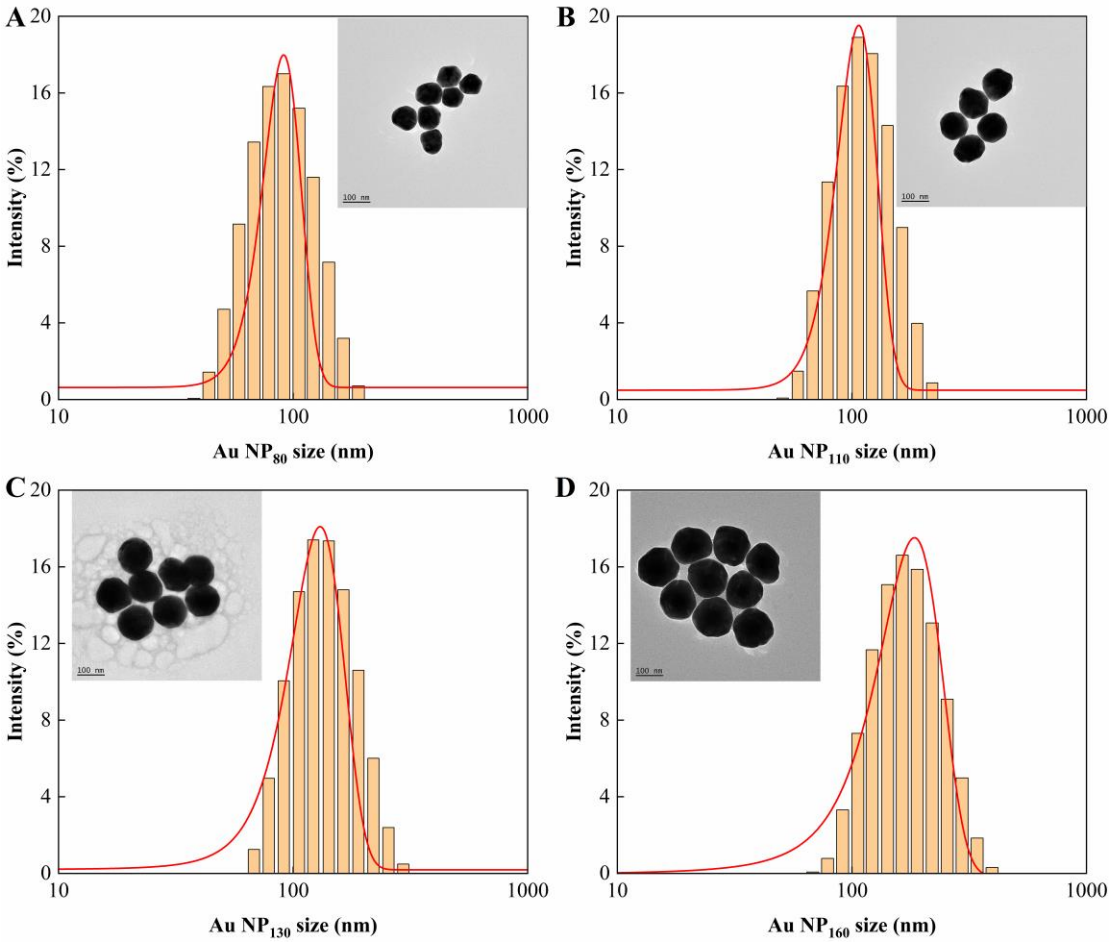

Fig. S2 The DLS size distribution diagram of the (A) Au NP_{80}, (B) Au NP_{110}, (C) Au NP_{130}, and (D) Au NP_{160}. Inserts are the corresponding TEM images.

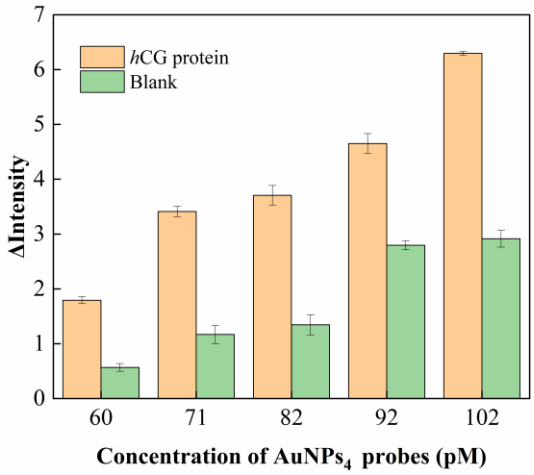

Fig. S3 The effect of the concentration of Au NP_{160} probes on *h*CG detection.

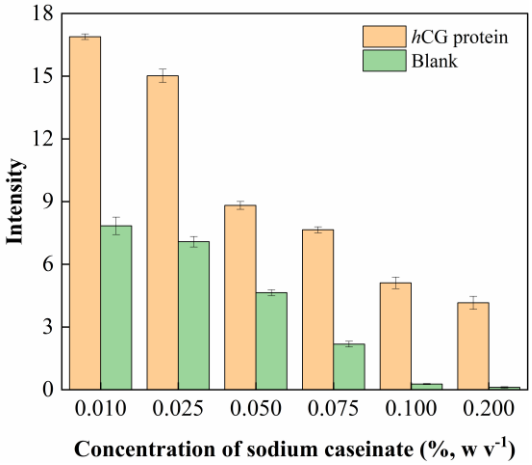

Fig. S4 The effect of the concentration of sodium caseinate on the detection of *h*CG protein.

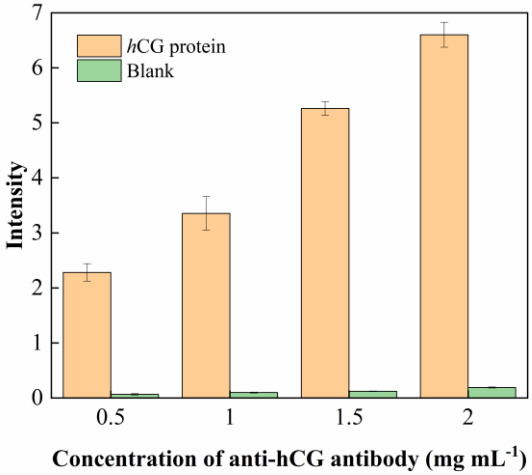

Fig. S5 The effect of concentration of anti-*h*CG capture antibody on the detection of *h*CG protein.

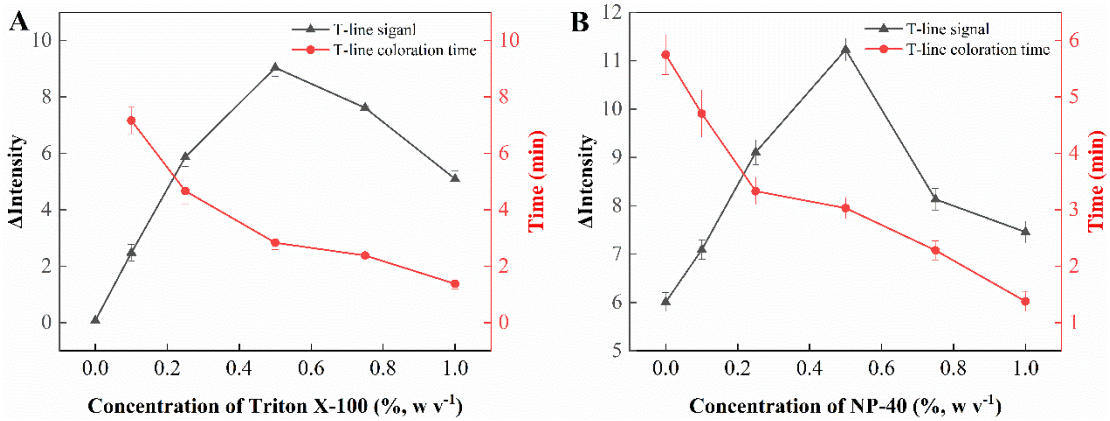

Fig. S6 Effects of the concentration of (A) Triton X-100 and (B) NP-40 on the detection of *h*CG protein and T-line coloration time.

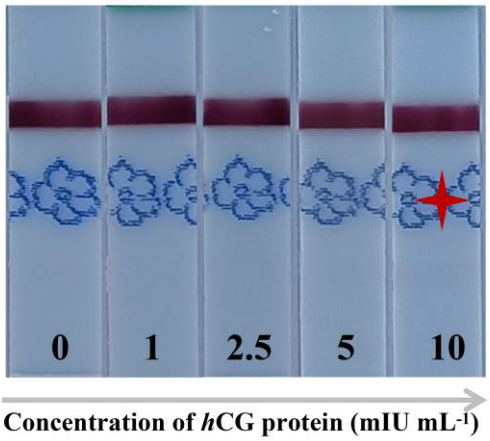

Fig. S7 Images of the commercial test strip on the detection of *h*CG protein.

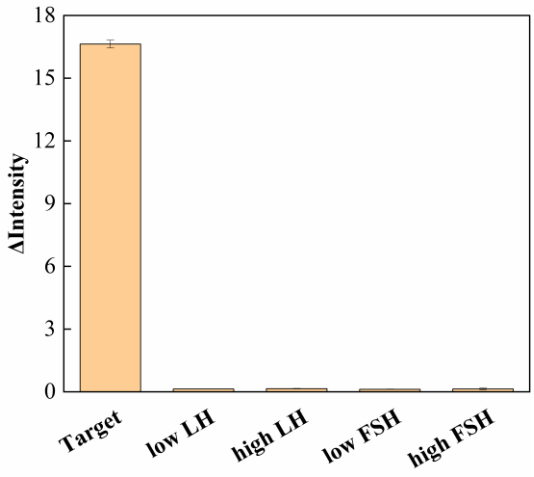

Fig. S8 The specificity of the signal enhanced-LFIA at room temperature.

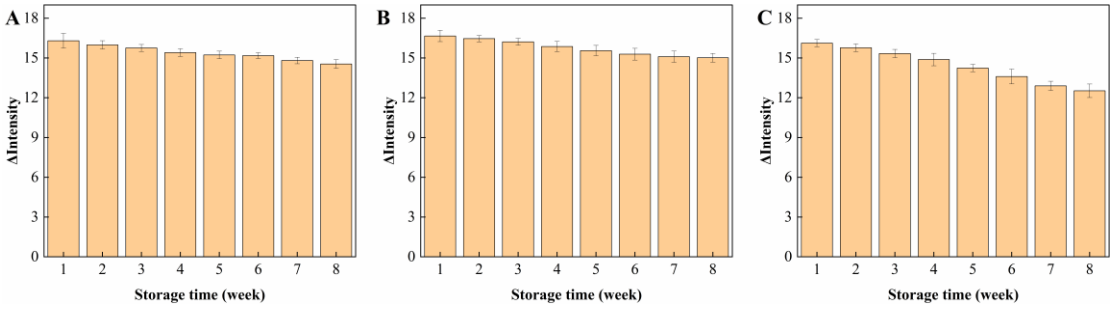

Fig. S9 Stability of the Au NPs-based sandwich-type LFIA at (A) 4 ℃, (B) room temperature and (C) 40 ℃.

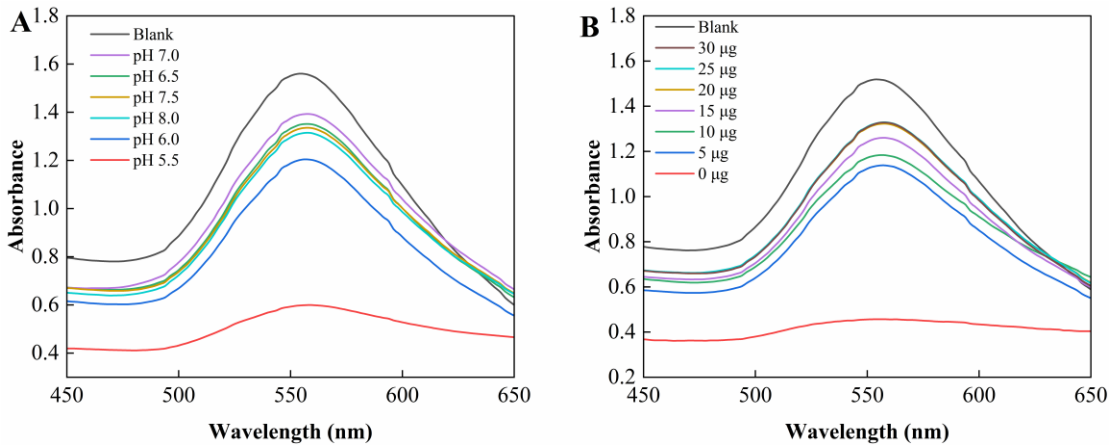

Fig. S10 Optimization of (A) pH and (B) *h*CG detection antibody concentration for prearation of Au NPs-conjugates.