Supporting Information

One-step *in Situ* Solid-substrate-based Whole Blood Immunoassay Based on FRET Between Upconversion and Gold Nanoparticles

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MATERIALS AND METHODS

Materials

All solvents and chemicals were of analytical or chemical pure grade and were used as received without further purification. All Immunoglobulin G (IgG) were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. The rabbit anti-goat IgG purified by protein A was polyclonal antibody, and there was only one cross-reaction antigen, namely goat antigen. Bovine serum albumin (BSA) was purchased from Dingguo Biotechnology Development (China). 50% Glutaraldehyde (GA) solution and Sodium cyanoborohydride were obtained from Aladdin. The rest chemical reagents were obtained from Sigma-Aldrich. The aqueous solutions were prepared using deionized water (Mill-Q, Millipore, 18.2 MΩ resistivity). The whole blood was obtained in the First Bethune Hospital of Jilin University from healthy volunteers, which was anti-coagulated by adding heparin, and stored at -20 °C. The glass slide was microscope slide purchased from Citotest Labware Manufacturing Co., Ltd. The thickness of the slide was 1.0-1.2 mm.

Apparatus

The size and morphology of the prepared UCNPs and GNPs, as well as the surface modification of the substrates were characterized by a JEM-2100F electron microscope operated at 200 kV and S-4800 Scanning Electron Microscope, respectively. X-ray diffraction (XRD) measurements were performed on a Bruker D8-advance XRD with Cu Kα irradiation (λ= 1.5418 Å) and 2θ range from 10° to 80°. Ultraviolet–Visible (UV-Vis) absorption spectrum was detected by a UV-3101 spectrophotometer. Fluorescence spectra were measured at room temperature (RT) on a Hitachi F-4500 fluorescence spectrofluorimeter, the excitation wavelength was 980 nm from a CW diode laser. The surface modification of the substrate with UCNPs was characterized by the Atomic Force Microscopy (Bruker Multimode 8). The AFM was operated in tapping mode at room temperature, a RTESP silicon probe with a

spring constant of ~40 N/m and a typical resonance frequency of ~300 kHz was used. The Fourier transform infrared (FT-IR) spectrum measurements were performed using VERTEX 70 FT-IR spectrometer with the KBr technique.

Synthesis and surface modification of $NaYF_4:Yb^{3+},Er^{3+}$ UCNPs

β-$NaYF_4$:20%Yb,2%Er UCNPs were synthesized according to previous report (Li and Zhang, 2008), and were dispersed in cyclohexane. In order to render UCNPs water soluble and allowing anchor of bio-molecules, a two-step approach was carried out to obtain amino-functionalized UCNPs (Bogdan et al., 2011). Briefly, 80 mg oleic acid coated $NaYF_4$: Yb^{3+}, Er^{3+} nanoparticles in 8 mL cyclohexane was added to 8 mL of 0.1 M HCl solution. After being stirred at room temperature overnight, the solution was centrifuged (11500 rpm, 4°C, 20 min) twice and washed with water, the ligand-free nanoparticles were obtained and dispersed in 12 mL deionized water. Subsequently, 170 mg of 50 wt% polyethyleneimine (PEI) was added into the above solution and stirred at room temperature for 24 h, the amino-modified nanoparticles were obtained after centrifugation (11500 rpm, 4 °C, 15 min) for three times and re-dispersed in 8 mL water.

Synthesis and IgG conjugation of GNPs

GNPs were synthesized by sodium citrate reduction method according to the literature with slight modification (Ji et al., 2007). Briefly, 1.0 mL of 1% $HAuCl_4$ was added into 50 mL of boiling water, after stirring for 5 min, 0.8 mL of 5% sodium citrate was added into the solution with continuous stirring vigorously. Then the reaction mixture was kept boiling for 1 h and cooled down to room temperature. Finally, the concentration of GNPs was adjusted to 0.1 mg /mL for further application.

GIgG-conjugated GNPs were prepared according to the literature (Wang et al., 2009). Typically, the pH of GNPs suspension was adjusted to 8.0 with 0.1 M K_2CO_3. Then 900 μg gIgG (20% more than the minimum amount, which was determined using a flocculation test) was added into 10 mL of pH-adjusted colloidal GNPs suspension followed by stirring mildly at room temperature for 1 h. After that, 1% of

BSA was added as blocking agents, and the reaction was continued for another 1 h. Finally, the suspension was centrifuged (10500 rpm, 4 °C, 15 min) three times to get rid of unbound gIgG and excess BSA. Afterwards the supernatant was removed and the soft sediment was re-suspended in 10 mL of 10 mM PBS (PH=7.4) for further use.

The flocculation test

The flocculation test was conducted according to the literature (Daumas et al., 2002). Briefly, an increasing volume (5, 15, 25, 35, 45, 60, 75, 90, 105 μL) of 1 mg/mL gIgG was added into 1 mL of GNPs solution. Then, adjusted the solutions to be 2 mL with deionized water and pH=8.0 with 0.1 M K_2CO_3. After being oscillated for 1 h at room temperature, 100 μl of 10% NaCl solution was added into the solution. Being incubated for 15 min, the absorption spectra were measured.

As shown in (Fig. S1), the absorption spectra present obvious broadening when the amount of gIgG (5, 15, 25, 35 μg) are not enough, which indicate the aggregation of GNPs. With further increasing the quantity of gIgG, the spectrum broadening disappear and the spectrum peaks begin to blue shift. When the amount of gIgG is 75 μg, the absorption spectrum maintain invariability. Therefore, 75 μg of gIgG is the minimal amount to stabilize 1 mL of GNPs, and 900 μg of gIgG (20% more than the minimum amount) is used to modify 10 mL of GNPs.

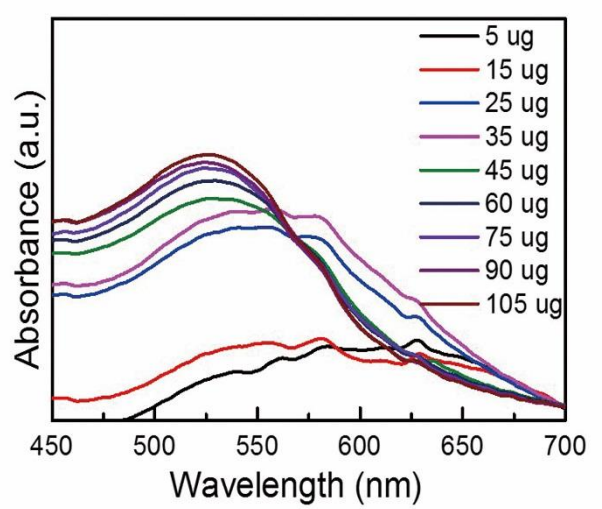

Fig. S1. Absorption spectra of GNPs modified with different amount of gIgG (5, 15, 25, 35, 45, 60, 75, 90, 105 μg) in the salt environment (10% NaCl solution).

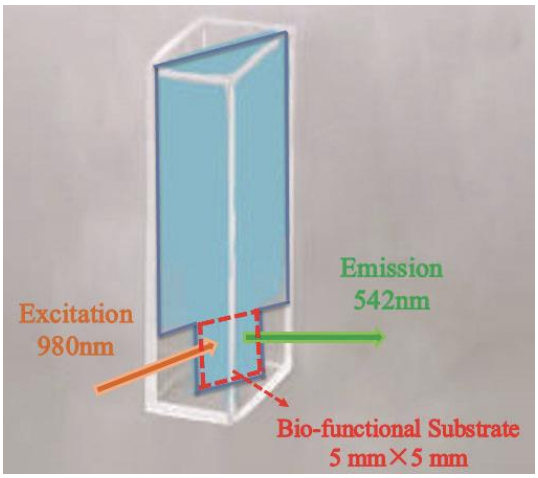

Fig. S2. The schematic diagram of the instrumental setup.

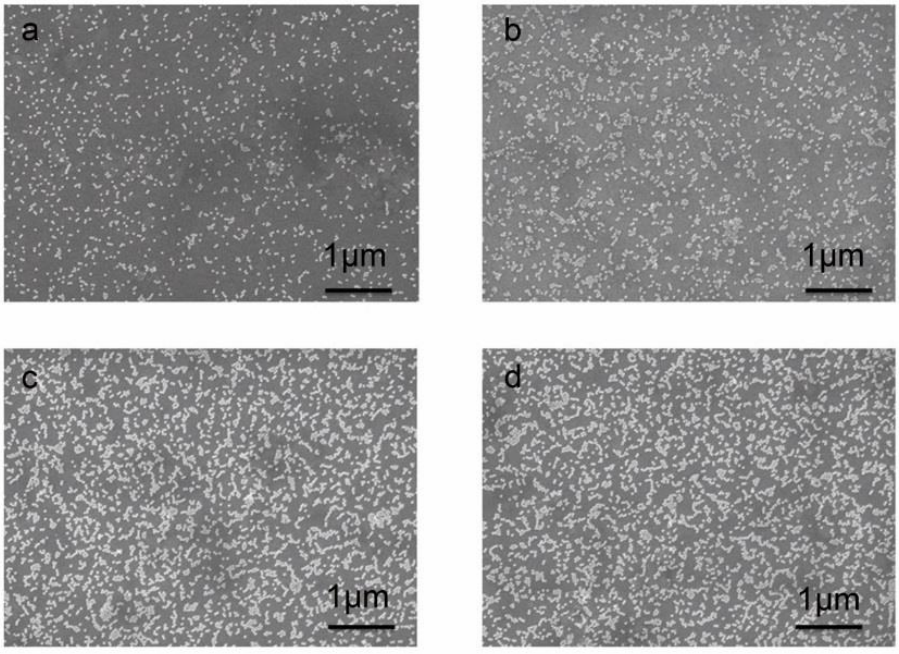

Fig. S3. SEM images of the silicon wafer substrates modified with UCNPs with different time, (a) 8 h, (b) 12 h, (c) 24 h and (d) 36 h.

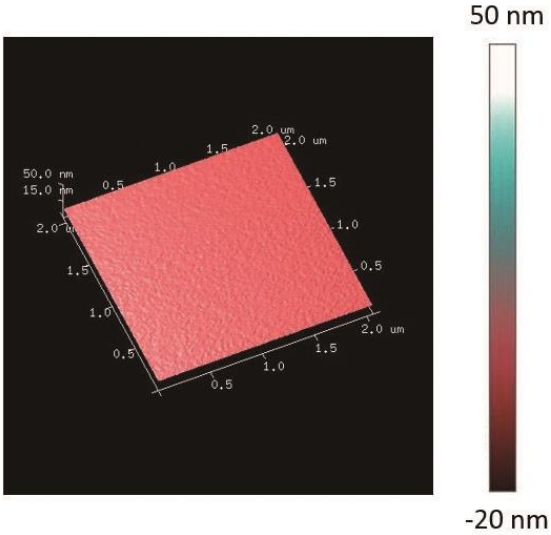

Fig. S4. AFM image of the bare substrate

The energy transfer mechanism of the immunoassay

Generally, the lifetime changes of the donor emission is a more direct and effective manner to characterize FRET. FRET is a typical non-radiative process which results in the emission lifetime change of the donor, while reabsorption is static in nature and has thus no effect on the emission lifetime of the donor. Considering that the dynamic of upconversion is modulated by energy transfer process which involves more electronic states, the down-conversion emission can reflect the de-population process of emitting energy state more precisely.

Since the UCNPs on the substrate were monolayerly distributed and the upconversion fluorescence was not strong enough to measure the lifetime changes before and after energy transfer. We simulated the combination of UCNPs and GNPs in solution. UCNPs were modified with rabbit-anti-goat IgG and GNPs with goat IgG, being same with that on the substrate. The fluorescence spectra of UCNPs and UCNPs-GNPs complex were detected under 980 nm excitation. The lifetime of 542 nm emission of UCNPs and UCNPs-GNPs complex under 488 nm excitation were recorded.

As shown in Fig. S5, the total quenching efficiency was calculated by $E=1-I/I_0$ to be 69% according to the upconversion fluorescence spectra. The FRET efficiency was calculated by $E=1-\tau/\tau_0$ to be 46.6% according to the lifetime changes (Lakowicz,

1999). Thus, it is obvious that the upconversion fluorescence quenching followed both reabsorption and FRET, although FRET is dominated.

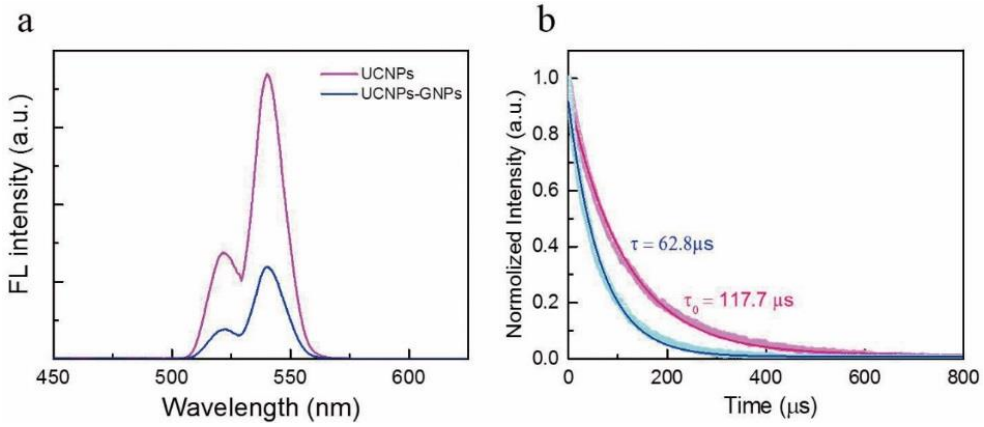

Fig. S5. (a) Upconversion fluorescence spectra of UCNPs (pink) and UCNPs-GNPs complex (blue) under 980 nm excitation; (b) the fluorescence decay curves of down-conversion emission monitored at 542 nm under 488 nm excitation and best-fitted curves for UCNPs (pink) and UCNPs-GNPs complex (blue)

The influence of the red-shift of modified GNPs on energy transfer efficiency was evaluated according to the overlap integral calculation (Lakowicz, 1999).

$$J(\lambda) = \int F_D(\lambda) E_A(\lambda) \lambda^4 d\lambda \qquad \text{Equ. S1}$$

Where $F_D(\lambda)$ and $E_A(\lambda)$ are the area normalized emission and absorption spectra of the donor and acceptor, respectively.

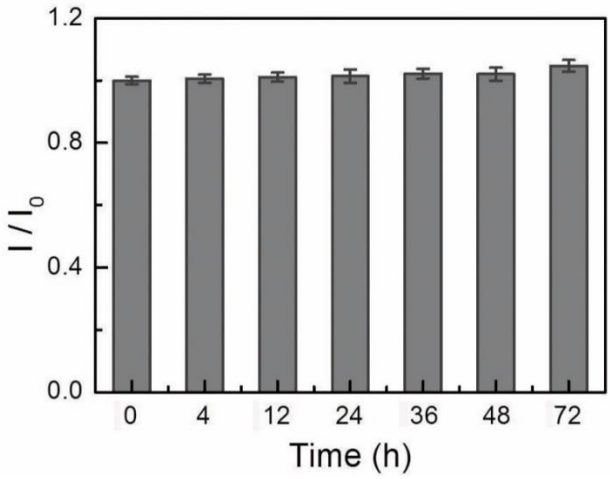

Fig. S6. The relative fluorescence intensity (I/I_0) of the bio-functional substrate after being kept in PBS under 4 ℃ for different times. I_0 and I represent the original

upconversion fluorescence intensity and the intensity after being kept for different times, respectively.

Analyte	Detection range	Reference
Goat IgG	0.09-60 μg/mL	our assays
Human IgG	0.05-2.5 μM	Liang, G.X. *et. al.*
Mouse IgG	0.1-20 mg/L	Ma, Q. *et. al.*
Goat antihuman IgG	3-67 μg/mL	Wang, M. *et. al.*
Mouse IgG	0.05-1.00 μg/mL	Do, J. *et. al.*

Table. S1. Reported detection range of some assays in buffers.

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