Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Upconversion fluorescence resonance energy transfer biosensor for sensitive detection of human immunodeficiency virus antibodies in human serum

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1. Experimental Section

Reagents and materials

Rare-earth chlorides including $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$ and $ErCl_3 \cdot 6H_2O$, NH_4F, NaOH, oleic acid, 1-octadecene, human serum albumin (HSA), Hemoglobin (HB), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) were all purchased from Sigma-Aldrich.1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[Methoxy-(Polyethyleneglycol)-2000] (ammonium salt) (DSPE-PEG-Methoxy) were purchased from Avanti Polar Lipids. Myoglobin and anti-HIV-1 gp120 antibody were purchased from Abcam. Immunoglobulin G (IgG), Immunoglobulin E (IgE), Immunoglobulin A (IgA), Immunoglobulin D (IgD), Immunoglobulin M (IgM) were purchased from Dingguo biotechnology Co. Ltd. (Beijing, China). KH_2PO_4, Na_2HPO_4 and NaCl were purchased from Sangon Biotech (Shanghai). The peptide DSPE-KNNNGGGRKRIHIGPGPAFYTT and the control peptide DSPE-KNNNGGGRKTKRNTNRRPQD were synthesized through succinic anhydride method by Apeptide Co., Ltd. (Shanghai, China). All the solutions were prepared using ultrapure water (>18.2 MΩ) produced by a Millipore Milli-Q water purification system (Billerica, MA, USA). Healthy human serum specimen was obtained from XiangYa School of Medicine, Central South University (Changsha, China).

Synthesis of oleic acid-coated $NaYF_4$: Yb, Er nanoparticles

Water-insoluble oleic acid-coated $NaYF_4$: Yb, Er nanoparticles (OA-UCNPs) were synthesized according to the method described by literature with slight modification.[S1] In brief, $YCl_3 \cdot 6H_2O$ (0.78 mmol), $YbCl_3 \cdot 6H_2O$ (0.20 mmol), and $ErCl_3 \cdot 6H_2O$ (0.02 mmol) (1 mmol, Y: Yb: Er =78%: 20%: 2%) were added to a 50 mL three-necked flask containing OA (6 mL) and 1-octadecene (15 mL). The reaction mixture was heated to 100 °C under vacuum with stirring for 30 min to remove residual water and oxygen and then heated to 160 °C for another 30 min to form a homogeneous solution and then cooled down to room temperature. Then, 10 mL of

methanol solution containing NaOH (2.5 mmol) and NH_4F (4 mmol) was added slowly and the resultant solution was stirred for an additional 30 min at 50 °C. The reaction mixture was heated to 100 °C under vacuum with stirring for 30 min to remove methanol and then was rapidly heated to 300 °C under stirring and kept at this temperature for 1 h under Ar protection and then cooled down to room temperature. The resulting nanoparticles were precipitated by the addition of ethanol, collected by centrifugation at 6000 rpm for 5 min, washed several times with ethanol and dried under vacuum for further experiments.

Preparation of the peptide-functionalizable UCNPs

Water-soluble and peptide-functionalizable UCNPs were prepared according to the literature method with minor modifications.[S2] Briefly, the OA-coated $NaYF_4$: 20%Yb, 2%Er UCNPs (1.0 mg) were added into a chloroform:methanol (v:v=3:1) mixture solution (2 mL) containing 10.0 mg of DSPE-PEG_{2000}-Methoxy and DSPE-peptide (mole ratio= 9:1) in a round-bottom flask (10 mL), and sonicated for 10 min. Then the mixture was dried in a rotary evaporator under reduced pressure at room temperature to form a lipid film on the inside wall of the flask. The lipid film was hydrated with ultrapure water (4 mL), and the UCNPs became soluble after vigorously sonication, which can be further stirred vigorously at 75 °C for 15 min to obtain colorless and transparent solution. The solution was transferred to a microtube. The large aggregates were discarded by centrifugation slightly (3000 rpm for 4 min), excess DSPE-PEG-Methoxy and DSPE-peptide were removed by centrifugation at 22000 rpm for 20 min, and washed three times with ultrapure water. The obtained peptide-functionalizable UCNPs were finally suspended in 1 mL of ultrapure water and stored at 4 °C for further experiments. The concentration of the peptide-UCNPs was calculated as ~ 1 mg mL^{-1}.

Preparation of graphene oxide (GO)

GO was prepared according to the method as described in our previous work with some modification.[S3] It was prepared by a modified Hummers method using graphite

powder as starting material, the GO suspension (~2 mg mL^{-1}) was sonicated in an ice-bath using a probe-type sonicator under a power of 40 W for 4 h (work 2 s, rest 4 s). The resulting suspension was centrifuged at 10,000 rpm for 30 min, and then discarded the sediments.

Characterization of UCNPs and GO

The morphologies of the nanoparticles were obtained using a JEOL JEM-2100 transmission electron microscope (TEM). Dilute colloid solutions of the OA-coated UCNPs dispersed in cyclohexane and peptide-UCNPs dispersed in water were drop-cast on thin, carbon formvar-coated copper grids respectively. Dilute colloid solutions of graphene oxide and peptide-UCNPs-GO complex in water were drop-cast on micro-mesh copper grids respectively and air dried before imaging. The X-ray diffraction (XRD) patterns of OA-coated UCNPs were performed on a Rigaku D/Max-Ra x-ray diffractometer using a Cu target radiation source (λ =0.14428 nm). The hydrodynamic size distribution of the OA-coated UCNPs and peptide-UCNPs were determined using a Malvern Zetasizer (Nano-ZS, USA). Fourier-transform infrared (FT-IR) spectrum analysis was performed with a Nicolet 4700 Fourier transform infrared spectrophotometer (Thermo Electron Co., USA) by using the KBr method. The UV-vis absorption spectrum of GO was recorded on a UV-2450 UV-vis spectrometer (Shimadzu, Japan).

Determination of the anti-HIV-1 gp120 antibody

For the determination of anti-HIV-1 gp120 antibody, a certain concentration of antibody solution and peptide-functionalizable UCNPs (final concentration of 35 μg mL^{-1}) were mixed in the assay buffer (50 mM phosphate, 137 mM NaCl, pH 7.4). The mixture was first incubated at 37 °C for 90 min, then GO (final concentration of 80 μg mL^{-1}) was added to the mixture and further incubated at 37 °C for 90 min. The upconversion fluorescence spectra of the final mixture were measured using a FluoroMax-4 Spectrofluorometer (HORIBA Jobin Yvon, Inc., NJ, USA) equipped with an external 980 nm diode CW laser (Changchun New Industries Optoelectronics

Tech. Co., Ltd.) as the excitation source instead of the internal excitation source.

2. Supplementary Figures and Table

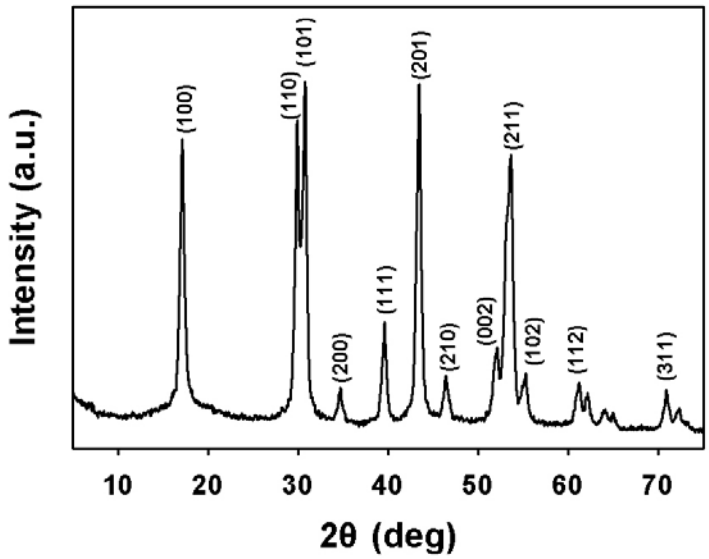

Fig. S1 XRD analysis of the UCNP nanocrystals (NaYF4: Yb: Er = 78%:20%:2%). The peak positions and intensities of the nanocrystals agreed well with the calculated values for the hexagonal NaYF4: Yb, Er (JCPDS no. 28-1192). No peaks of any other phase were detected, indicating the high purity of the final product.

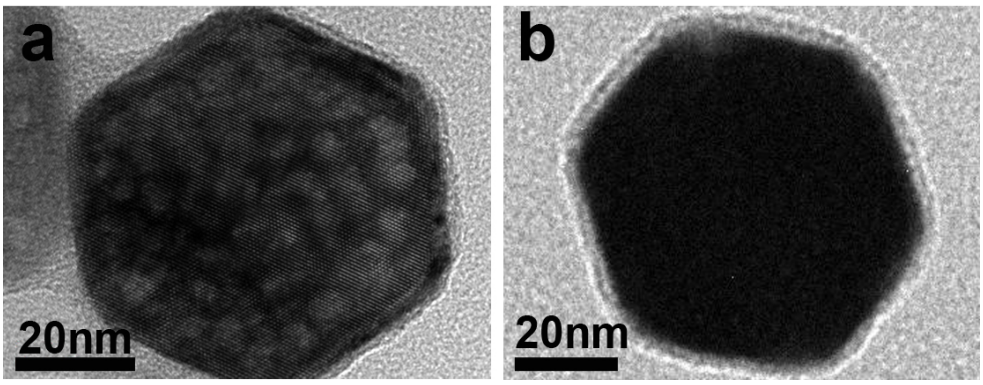

Fig. S2 High-resolution TEM images of the (a) as-prepared OA-coated UCNPs and (b) peptide-functionalizable UCNPs. In comparison with the OA-coated UCNPs, the peptide-functionalizable UCNPs showed the core/shell nanocrystals with a uniform, approximately 2 nm thick, hydrophobic oleic acid/lipid layer around the surface.

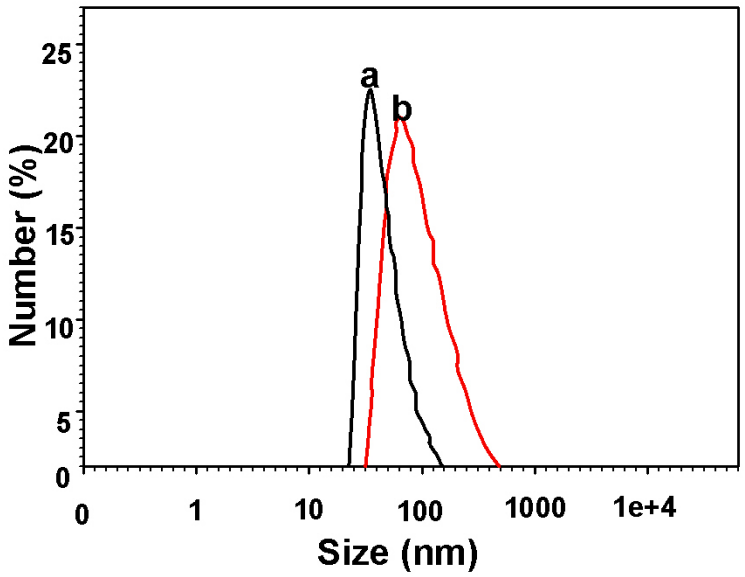

Fig. S3 Hydrodynamic size distribution of (a) OA-coated UCNPs in cyclohexane and (b) peptide-functionalizable UCNPs in water, determined by dynamic light scattering. The peptide-functionalizable UCNPs were well-dispersed in water with a mean hydrodynamic diameter of about 90 nm. In comparison with OA-coated UCNPs dispersed in cyclohexane (ca. 65 nm), this increase of approximately 25 nm in diameter was in agreement with a monolayer of the PEGylated phospholipids.

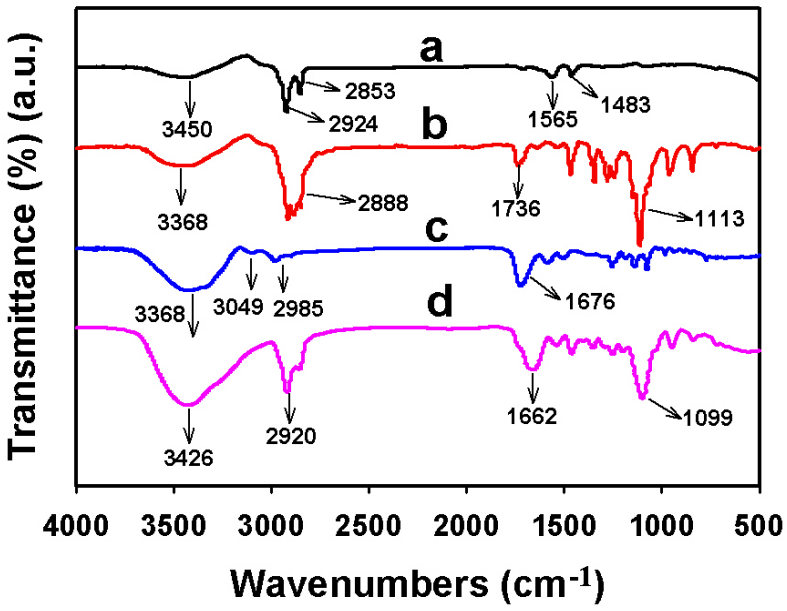

Fig. S4 FT-IR spectra of the (a) as-prepared OA-coated UCNPs, (b) DSPE-PEG-Methoxy phospholipid, (c) DSPE-peptide conjugation, and (d) peptide-functionalizable lipid-UCNPs. A band appeared around 1099 cm^{-1} for the peptide-functionalizable lipid-UCNPs, which was not observed for the UCNPs coated with oleic acid. This band was ascribed to the stretching vibrations of the C-O bond in PEG. The absorption band around 1565 cm^{-1} for the UCNPs coated with oleic acid, attributed to the stretching vibration of carboxyl group in oleic acid, was shifted to 1662 cm^{-1} for the peptide-functionalizable lipid-UCNPs, corresponding to the stretching vibration of amide bond in DSPE-PEG-Methoxy phospholipid and DSPE-peptide conjugation. The absorption bands around 2920 cm^{-1} slightly increased in the peptide-functionalizable lipid-UCNPs as compared to those coated with oleic acid, attributed to the increased amount of CH_2 groups in the PEG coating. The UCNPs, either coated with oleic acid or DSPE-PEG-Methoxy phospholipid, exhibited a broad band around ~3450 cm^{-1}, corresponding to the O-H stretching vibration.

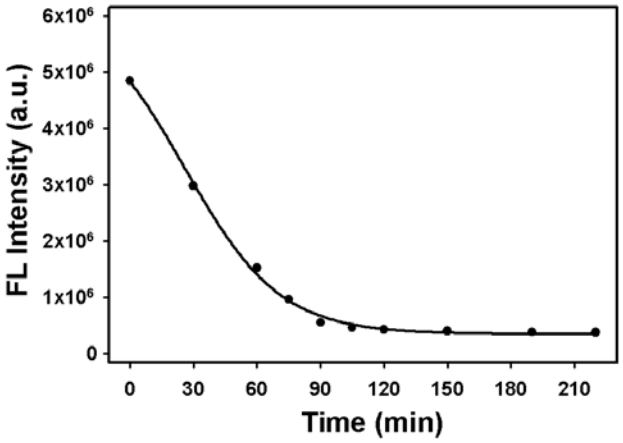

Fig. S5 Effect of the incubation time of peptide-UCNPs with GO on the upconversion fluorescence quenching. The concentrations of peptide-UCNPs and GO were 35 μg mL^{-1} and 80 μg mL^{-1}, respectively. All experiments were performed in PBS buffer (50 mM phosphate, 137 mM NaCl, pH 7.4).

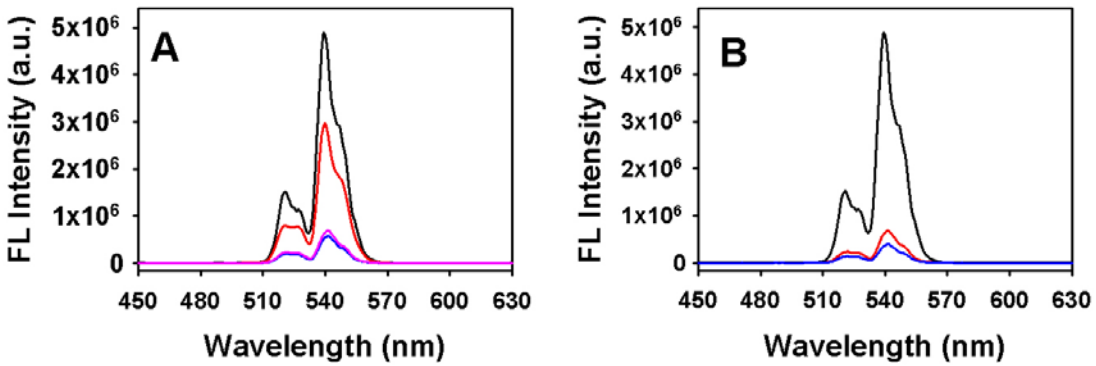

Fig. S6 (A) Upconversion fluorescence spectra of peptide-functionalizable UCNPs (black curve), peptide-functionalizable UCNPs + GO (blue curve), peptide-functionalizable UCNPs + anti-HIV-1 gp120 antibody + GO (red curve), and peptide-functionalizable UCNPs + human serum albumin (HSA) + GO (purple curve). (B) Upconversion fluorescence spectra of control peptide-modified UCNPs (black curve), control peptide-modified UCNPs + GO (blue curve), and control peptide-modified UCNPs + anti-HIV-1 gp120 antibody + GO (red curve). The concentrations of the peptide-functionalizable UCNPs and control peptide-modified UCNPs were both 35 μg mL^{-1}. The concentrations of GO and anti-HIV-1 gp120 antibody or HSA were 80 μg mL^{-1} and 150 nM, respectively. The sequence of the control peptide was DSPE-KNNNGGGRKTKRNTNRRPQD. The adsorption of the control peptide-modified UCNPs on GO might be attributed to hydrophobic interaction and electrostatic interaction between six positively charged amino acids (RK…KR…RR…). Moreover, the presence of multiple control peptides on the UCNPs could also generate multivalent interactions with GO so as to mediate efficient adsorption of the control peptide-modified UCNPs onto GO.

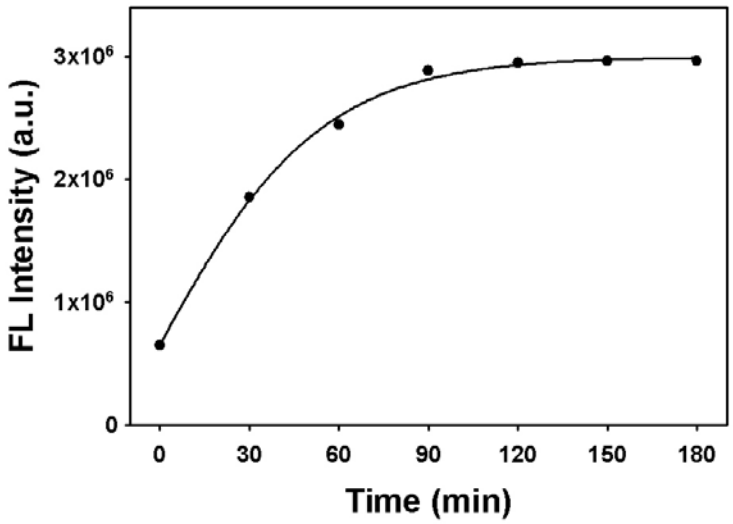

Fig. S7 Effect of the interaction time between peptide-functionalizable UCNPs and antibody on the fluorescence intensity. The concentrations of the peptide-functionalizable UCNPs and anti-HIV-1 gp120 antibody were 35 μg mL^{-1} and 150 nM, respectively. After the incubation of peptide-UCNPs with antibody, the mixture was incubated further 90 min after adding 80 μg mL^{-1} GO.

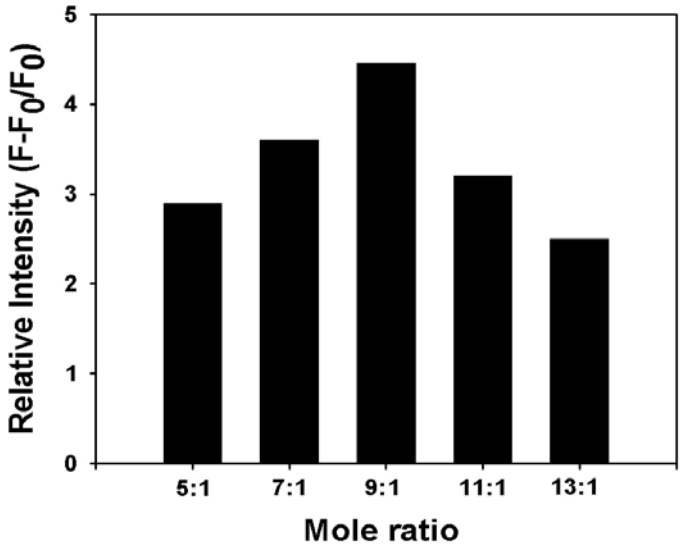

Fig. S8 The influence of the mole ratio of DSPE-PEG2000-Methoxy to DSPE-peptide on the performance of the biosensor. The mole ratio of 9:1 was optimal in terms of the best signal-to-background ratio. When a larger mole ratio was used, which indicated that the number of the antigenic peptide on the surface of per UCNPs was relatively little, the quenching efficiency of a fixed amount of GO would decrease, leading to a relatively high background. In contrast, when a smaller mole ratio was used, the increasing antigenic peptide on the surface of UCNPs would consume more antibodies, resulting in a low responsive signal.

The concentrations of the peptide-functionalizable UCNPs, anti-HIV-1 gp120 antibody and GO were 35 μg mL^{-1}, 150 nM and 80 μg mL^{-1}, respectively.

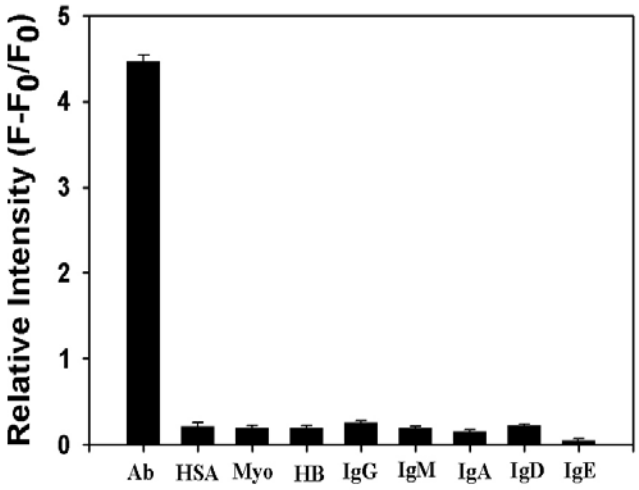

Fig. S9 Relative fluorescence intensity of the upconversion FRET-based biosensor toward anti-HIV-1 antibody and some other proteins. Ab, anti-HIV-1 gp120 antibody; HSA, human serum albumin; Myo, myoglobin; HB, hemoglobin; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; IgD, immunoglobulin D; IgE, immunoglobulin E. The concentrations of all other proteins were 1.5 μM. The concentrations of the antibody, peptide-UCNPs and GO were 150 nM, 35 μg mL^{-1} and 80 μg mL^{-1}, respectively. The error bars represented standard deviation of three repetitive experiments.

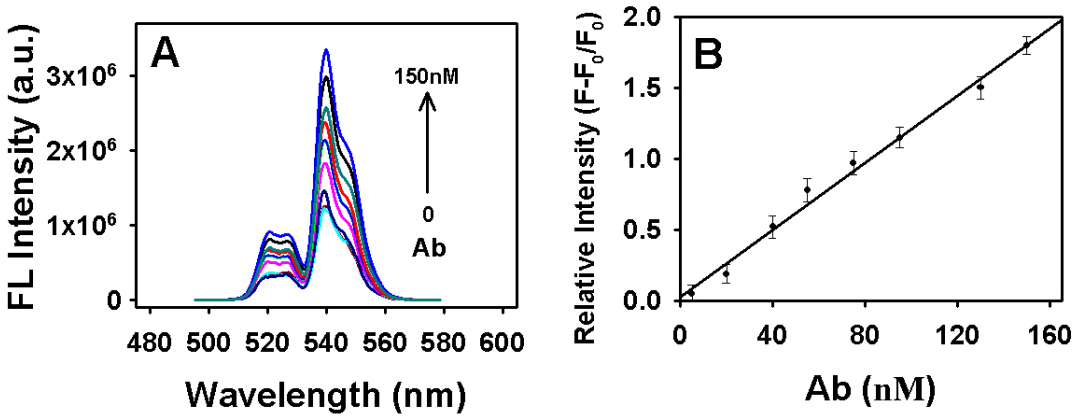

Fig. S10 (A) Upconversion fluorescence spectra of the biosensor with varying concentrations of antibody (0, 5, 20, 40, 55, 75, 95, 130 and 150 nM). (B) Linear relationship between the fluorescence relative intensity and the concentrations of antibody within the range of 5-150 nM. All experiments were performed in human serum 10-fold diluted with PBS buffer (50 mM phosphate, 137 mM NaCl, pH 7.4). The slightly increased background in the assay for 10-fold diluted human serum was attributed to non-specific adsorption of serum proteins on GO surface, which might affect the interaction between the GO and the peptide decorated on the surface of UCNPs.

Table S1 Analytical results of the anti-HIV-1 gp120 antibody in human serum using the upconversion FRET-based biosensor.

sample	added (nM)	found (nM)	recovery	RSD (n=3)
1	25	23.5	95%	6.1%
2	55	59.5	108%	5.9%
3	80	77.5	97%	4.8%
4	105	109.8	105%	5.2%

3. References

S1 Z. Li and Y. Zhang, *Nanotechnology*, 2008, **19**, 345606.

S2 L. L. Li, R. Zhang, L. Yin, K. Zheng, W. Qin, P. R. Selvin and Y. Lu, *Angew. Chem. Int. Ed.*, 2012, **51**, 6121-6125.

S3 H. Wang, Q. Zhang, X. Chu, T. Chen, J. Ge and R. Yu, *Angew. Chem. Int. Ed.*, 2011, **50**, 7065-7069.