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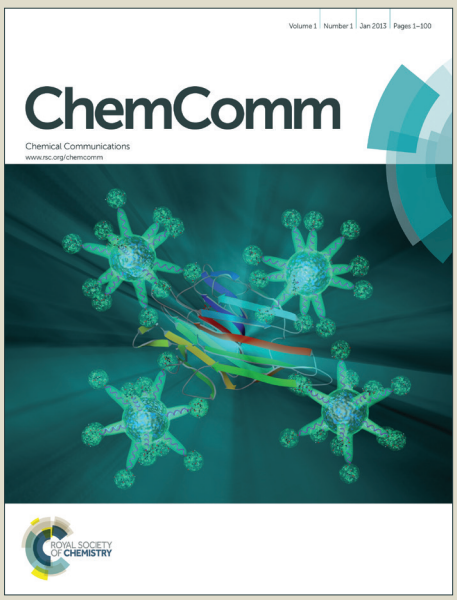

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COMMUNICATION

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

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A facile one-step approach was proposed to make hydrophilic and peptide-functionalizable upconversion nanoparticles (UCNPs), which was used to design a biosensor for the sensitive and selective determination of human immunodeficiency virus antibody in human serum based on FRET from UCNPs to the graphene oxide.

Sensitive and selective detection of molecular biomarkers in complex biological samples represents an essential approach in proteomics and clinical diagnostics. Currently, various fluorescence resonance energy transfer (FRET) between emerging nanomaterials and biomolecular recognition units has shown great potential for the development of novel clinic diagnostic techniques.[1] However, traditional downconversion nanomaterials and organic dyes commonly used as donors in the FRET process generally need photoexcitation in ultraviolet/blue region, in which region the endogenous chromophores in biological or environmental samples can also be excited, leading to strong background fluorescence and thus restricting their applications in complex biological samples.[2]

Lanthanide-doped upconversion nanoparticles (UCNPs), which are capable of emitting strong visible fluorescence under the excitation of near-infrared (NIR) light (typically ca. 980 nm), have attracted considerable attention. These UCNPs have been proved to exhibit superior features, such as improved quantum yield, tunable multicolor emission, minimal photobleaching, reenforced light penetration depth in tissue and low toxicity.[3] Most importantly, under the excitation of NIR light, the autofluorescence from biological samples and scattering light become negligible. These merits make UCNPs an ideal candidate as the donor in the FRET-based assay. Up to now, many research works have been reported to detect ions,[4] small molecules,[5] protein[6] and nucleic acids[7] based on FRET from UCNPs to various types of acceptors including organic dyes, gold nanoparticles, graphene and carbon nanoparticles.

To develop the UCNPs-based biosensor, a major challenge is to make water-dispersible, biocompatible, and functionalizable UCNPs, because they are normally prepared in organic solvents and capped with hydrophobic ligands.[8] Silica coating formed on the surface of UCNPs has been developed to render the UCNPs dispersible in water, but attenuated luminescence intensity and the requirement of further surface modification make this approach undesirable for bioassay.[9] Moreover, although direct synthesis of water-dispersible UCNPs or replacement of hydrophobic ligands with small hydrophilic ligands by ligand exchange could also make UCNPs both water-dispersible and functionalizable,[10] there is still a need for a simple and general method for producing biocompatible UCNPs. Recently, Lu's group reported an approach to engineer the UCNP surface coating with a monolayer of functional phospholipids.[11] Despite the significant advantages, the conjugation of biomolecules with the phospholipids with active groups is still required.

Here we developed a facile one-step approach to make water-dispersible and peptide-functionalizable UCNPs through the self-assembly of phospholipid-peptide conjugation onto hydrophobic UCNPs surface, and designed a biosensor based on FRET from the peptide-functionalizable UCNPs to the graphene oxide (GO), a highly efficient energy transfer acceptor.[12] To demonstrate the utility of this strategy, we chose the human immunodeficiency virus (HIV) antibody assay as the model system. HIV is a well-known infection of the human immune system cells lentivirus. Until now, sensitive and selective determination of HIV antibody in blood samples is still a challenging work because of the high complexity of the sample matrix.

The principle of the upconversion FRET-based biosensor was shown in Scheme 1. An antigenic peptide from HIV type 1 (HIV-1) glycoprotein gp120 immunodominant regions was selected as recognition unit for the detection of anti-HIV-1 gp120 antibody, and the sequence is as follows: RKRIHIGPGPAFYTT.[13] A linker sequence KNNNGGG was added to spatially separate the peptide from the nanoparticles and enhance the accessibility to target HIV antibody. The polypeptide was first conjugated with the phospholipid to form phospholipid-peptide conjugation, which, as well as PEGylated phospholipid, was then used to prepare the peptide-functionalizable UCNPs through a one-step assembly driven by the hydrophobic van der Waals interactions between the hydrophobic tail of the phospholipids and the primary oleate ligands on the UCNP surface. Such a process not only makes

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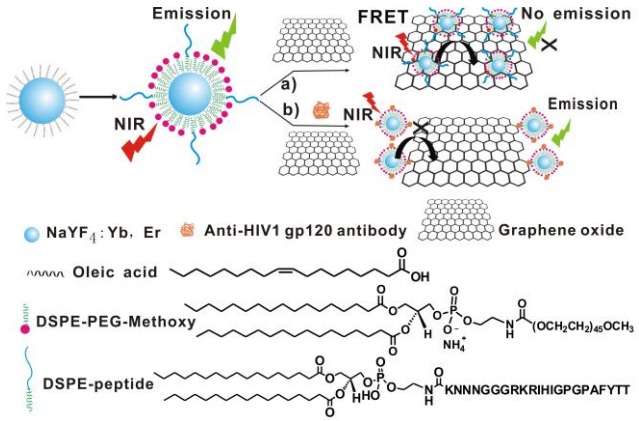

Scheme 1 Schematic illustration of the upconversion FRET-based biosensor for anti-HIV-1 gp120 antibody detection.

these nanocrystals well dispersible in water with excellent stability, but also avoids the conjugation of peptide with the phospholipid-modified UCNPs. The high flexibility of PEG molecules allows large molecules and nanomaterials to diffuse into the PEG-modified layer to interact with the ligands. When adding GO, peptide-functionalizable UCNPs could adsorb on the surface of GO via π-π stacking interactions and hydrophobic interactions between the peptides and GO. The upconversion fluorescence could be completely quenched through energy-transfer or electron-transfer processes. In contrast, in the presence of anti-HIV-1 gp120 antibody, the peptides preferred to bind to their antibody, leading to the formation of peptide-antibody complexes. Therefore the interactions between the peptides and GO would be inhibited, which kept the UCNPs far away from the GO surface, resulting in the decrease in quenching efficiency and the recovery of upconversion fluorescence.

Highly efficient upconverting $NaYF_4$: Yb, Er nanoparticles were synthesized using oleic acid (OA) as the stabilizing agent. The transimission electron microscopy (TEM) image shows that these nanoparticles display uniform hexagonal plate-like morphology with mean sizes of approximately 60 nm (Fig. 1A). The X-ray diffraction (XRD) analysis agrees well with that of pure hexagonal phase $NaYF_4$ nanocrystals (Fig. S1, ESI†). After functionalized with a mixture of PEGylated phospholipid and phospholipid-peptide conjugation, the resulting lipid-UCNPs show the core/shell nanocrystals with a uniform, approximately 2 nm thick, hydrophobic oleic acid/lipid layer around the surface (Fig. 1B and Fig. S2, ESI†). Dynamic light scattering (DLS) measurements indicate that the lipid-UCNPs are well-dispersed in water with a mean hydrodynamic diameter of about 90 nm. In comparison with oleic UCNPs dispersed in cyclohexane (ca. 65 nm), this increase of approximately 25 nm in diameter is in agreement with a monolayer of the PEGylated phospholipids (Fig. S3, ESI†).[11] In addition, the FT-IR spectra also demonstrate that the PEGylated phospholipid and phospholipid-peptide conjugation have been assembled successfully on the UCNP surface (Fig. S4, ESI†).

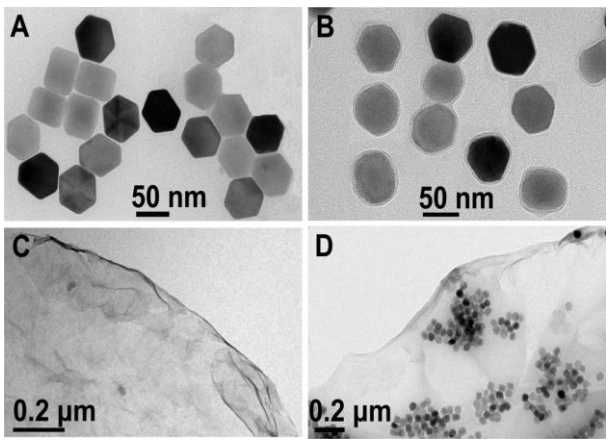

Fig. 1 TEM images of the (A) OA-coated UCNPs in cyclohexane, (B) peptide-functionalizable UCNPs in water, (C) as-prepared GO and (D) peptide-functionalizable UCNPs-GO complexes in water.

Compared with that of OA-coated UCNPs, the peptide-functionalizable UCNPs gave a slightly decreased upconversion fluorescence spectrum with two emission bands at 540 and 650 nm respectively, which overlapped with the absorption spectrum of GO as shown in Fig. 2A. The strong fluorescence of peptide-functionalizable UCNPs was sufficiently quenched (~90%) after incubation with GO. In contrast, the interaction between GO and UCNPs modified with only PEGylated phospholipid resulted in ~8% fluorescence quenching under the identical conditions (Fig. 2B). This result indicated that the remarkable fluorescence quenching of peptide-UCNPs was attributed to the selective adsorption of peptides on UCNPs to the GO surface. The TEM image shown in Fig. 1D also confirmed the formation of peptide-UCNPs-GO complexes. The effect of GO concentration on the fluorescence quenching was investigated. As shown in Fig. 2C, the upconversion fluorescence of peptide-functionalizable UCNPs was gradually quenched with increased amounts of GO, and the quenching efficiency reached a plateau when the GO concentration was higher than 80 μg mL^{-1} (Fig. 2D). So, the GO

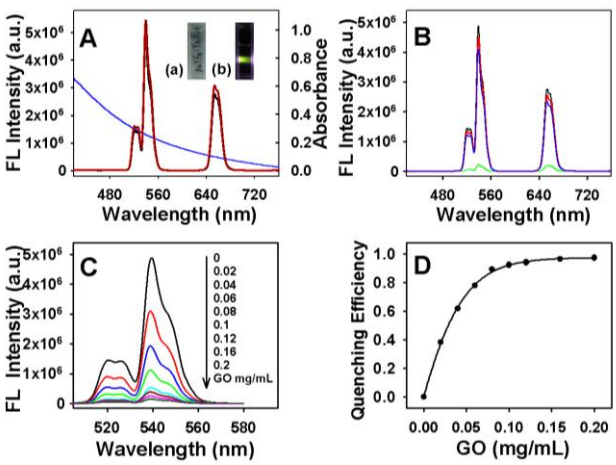

Fig. 2 (A) Upconversion fluorescence spectra of the OA-coated UCNPs (red curve), peptide-functionalizable UCNPs (black curve), and the absorption spectrum of GO (blue curve). (The inset shows a photograph of the peptide-UCNPs in water under ambient light (a) and irradiated by 980 nm light (b)). (B) Fluorescence spectra of peptide-functionalizable UCNPs before (black curve) and after (green curve) incubation with GO, and UCNPs modified with only PEGylated phospholipid before (red curve) and after (blue curve) incubation with GO. (C) Fluorescence spectra of peptide-UCNPs after incubation with various concentrations of GO. (D) Plot of fluorescence quenching efficiency versus GO concentration.

concentration of 80 μg mL^{-1} was selected for the subsequent experiments. In addition, the incubation time of peptide-UCNPs with GO was also studied (Fig. S5, ESI†), and an incubation time of 90 min was used in the subsequent experiments.

The detection of anti-HIV-1 gp120 antibody was then performed. As shown in Fig. S6A, ESI†, the incubation of HIV-1 antibody and peptide-functionalizable UCNPs indeed resulted in the fluorescence increase. In contrast, when human serum albumin was added to replace antibody or the control peptide-modified UCNPs was used, no obvious fluorescence increase was observed, indicating that the specific interaction between antigenic peptide and antibody could decrease the adsorption of peptide-UCNPs on the GO surface. The effect of interaction time between peptide-UCNPs and antibody on the fluorescence intensity was investigated (Fig. S7, ESI†), and a reaction time of 90 min was selected. The mole ratio of PEGylated phospholipid to phospholipid-peptide conjugation was also optimized as 9:1 in terms of the best signal-to-background ratio (Fig. S8, ESI†). Under the optimal conditions, the upconversion fluorescence intensity increased with the increasing antibody concentration (Fig. 3A), and the relative fluorescence intensity was linearly related to the antibody concentration in the range from 5 nM to 150 nM (Fig. 3B). The detection limit of antibody was calculated to be 2 nM according to the 3σ rule.

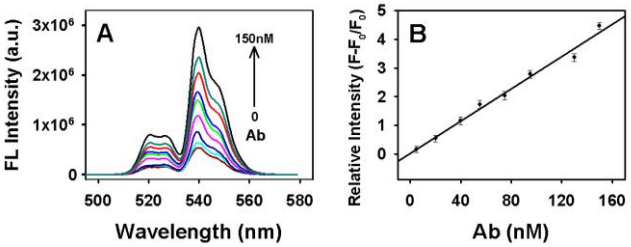

Fig. 3 (A) Upconversion fluorescence spectra of the biosensor with varying concentrations of antibody (0, 5, 20, 40, 55, 75, 95, 130 and 150 nM). (B) Linear relationship between the fluorescence relative intensity and the concentrations of antibody within the range of 5-150 nM. The error bars represented the standard deviations of three independent experiments.

To assess the specificity of the upconversion FRET-based biosensor for target antibody, the responses of the biosensor toward some other proteins were investigated. As shown in Fig. S9, ESI†, the other proteins did not generate significant signals, indicating that other proteins could not interact with peptide. In addition, the results implied that the high concentrations of proteins did not interfere with the binding of peptide-UCNPs to GO, which may be attributed to much weaker adsorption of these proteins on GO than that of peptide-UCNPs to GO due to the multivalent interaction of multiple epitope peptides on the UCNP surface with GO. The results clearly demonstrated the excellent specificity of the biosensor for the target antibody.

To investigate the ability of the upconversion FRET-based biosensor to overcome the interference from background fluorescence and scattering light, we applied the developed biosensor to spiked serum samples. With 10-fold diluted human serum as the assay medium, the same antibody-dependent fluorescence changes as that in aqueous buffer were observed (Fig. S10A, ESI†) except for a slightly increased background. A linear range from 5 to 150 nM was also obtained in the diluted serum (Fig. S10B, ESI†). In addition, the recovery experiment was also performed. Four human serum samples spiked with different concentrations of anti-HIV-1 gp120 antibody were determined, and the results were shown in Table S1, ESI†. The recoveries were from 95% to 108% with RSD around 6%, which are acceptable for quantitative assays performed in biological samples.

In conclusion, we developed a facile one-step approach to make peptide-functionalizable UCNPs through the self-assembly of phospholipid-peptide conjugation onto UCNPs surface, and designed a biosensor based on FRET from the peptide-functionalizable UCNPs to graphene oxide. The sensor can be used for anti-HIV-1 gp120 antibody sensing both in an aqueous buffer and in a serum matrix with comparable performances, proving that the biosensor is capable of overcoming background interference from complex biological samples. The sensor was applied to determine antibody concentration in human serum with satisfactory results. Owing to the facile fabrication, the sensor could be readily developed to build up sensing platforms for various targets by self-assembly of different peptide to UCNPs.

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