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Fluorescence Resonance Energy Transfer Inhibition Assay for α-Fetoprotein Excreted during Cancer Cell Growth Using Functionalized Persistent Luminescence Nanoparticles

Bo-Yue Wu,[†] He-Fang Wang,[†] Jia-Tong Chen,[‡] and Xiu-Ping Yan*[,†]

[†]Research Center for Analytical Sciences, College of Chemistry, and [‡]Department of Biochemistry and Molecular Biology, College of Life Sciences, Nankai University, Tianjin 300071, China

Supporting Information

ABSTRACT: Persistent-luminescence nanoparticles (PLNPs) are promising as a new generation of photoluminescent probes for detection of biomolecules and bioimaging. Here we report a fluorescence resonance energy transfer (FRET) inhibition assay for α-fetoprotein (AFP) excreted during cancer cell growth using water-soluble functionalized PLNPs based on Eu^{2+}- and Dy^{3+}-doped $Ca_{1.86}Mg_{0.14}ZnSi_2O_7$. Polyethyleneimine-coated PLNPs were conjugated with AFP-antibody-coated gold nanoparticles as a sensitive and specific persistent photoluminescence probe for detection of AFP in serum samples and imaging of AFP excreted during cancer cell growth. Such PLNPs do not contain toxic heavy metals. Their long-lasting afterglow nature allows detection and imaging without external illumination, thereby eliminating the autofluorescence and scattering light from biological matrixes encountered under in situ excitation.

Fluorescent probes are increasingly used for biomarker detection and provide remarkable results in early detection, which is a crucial element for the timely diagnosis and successful treatment of all human cancers.[1] Development of more efficient and ultrasensitive fluorescent materials is becoming a forceful trend. However, fluorescent techniques often suffer limitations, such as a poor signal-to-noise ratio from tissue autofluorescence under external illumination and tissue damage or cell death due to irradiation using short-wavelength excitation light.[2]

Persistent-luminescence nanoparticles (PLNPs) are one of the important light−light transformation and energy-saving materials. These materials have found extensive applications in many fields, such as traffic signs, interior decoration, and light sources.[3,4] The long-lasting afterglow of PLNPs can last several hours and can be optically excited before bioanalytical applications. Thus, the signal-to-noise ratio can be significantly improved because of the removal of the background noise originating from in situ excitation.[5] This property makes PLNPs particularly useful in photoluminescence (PL) detection and real-time monitoring in bioanalysis. Scherman and co-workers[5] pioneered the application of PLNPs in bioimaging and functionalized their PLNPs to make them water-soluble and influence their targeting. Chen and Zhang[6] employed PLNPs to enable simultaneous radiation and photodynamic therapies for cancer treatment. However, to the best of our knowledge, no attempts to employ PLNPs as PL probes for detection of biomolecules have been made, and reports of applications of PLNPs in bioimaging have been rare to date, probably because of the chemical inertness of PLNPs toward most target molecules[6] or the difficulty in determining the depth because of the lack of in situ excitation.

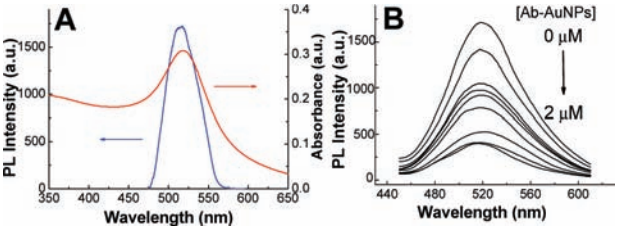

Figure 1. (A) Photoluminescence emission spectra of PEI-PLNPs (blue curve) and absorption spectra of Ab-AuNPs (red curve). (B) Quenching effect of Ab-AuNPs on the photoluminescence emission of PEI-PLNPs.

Here we report a fluorescence resonance energy transfer (FRET) inhibition assay for α-fetoprotein (AFP) excreted during cancer cell growth using water-soluble functionalized PLNPs. We have employed Eu^{2+}- and Dy^{3+}-doped $Ca_{1.86}Mg_{0.14}ZnSi_2O_7$ nanoparticles as an example of PLNPs because their PL emission spectra have maximum overlap with the absorption spectra of AFP-antibody−gold nanoparticle conjugates (Ab-AuNPs), resulting in maximum FRET efficiency (Figure 1A). AFP is the serum biomarker of hepatocellular carcinoma (HCC), which is the sixth most common cancer worldwide in terms of numbers of cases and has almost the lowest survival rates because of its very poor prognosis. Serum levels of AFP often increase under conditions such as periods of rapid liver cancer cell growth, cirrhosis, and chronic active hepatitis as well as carbon tetrachloride intoxication. Therefore, detection of serum levels of AFP can lead to early diagnosis of HCC.[7]

To probe AFP with high selectivity and sensitivity, we designed an inhibition assay[8] based on the modulation of FRET between polyethyleneimine (PEI)-capped PLNPs (PEI-PLNPs) and Ab-AuNPs via inhibition of the interactions between PEI-PLNPs and Ab-AuNPs by AFP (Scheme 1). To this end, a sol−gel procedure in combination with microwave irradiation was employed to prepare Eu^{2+}- and Dy^{3+}-doped $Ca_{1.86}Mg_{0.14}ZnSi_2O_7$ nanoparticles in a rapid way [Figures S1 and S2 in the Supporting Information (SI)]. The PL of as-prepared PLNPs lasted 6 h and became stable in a period of 30−180 min after

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Scheme 1. Schematic Illustration of the FRET Inhibition Assay for AFP Based on the PL Quenching of PEI-PLNPs by Ab-AuNPs

excitation (Figure S1B). PEI was then grafted onto the surface of the as-prepared PLNPs to enhance the biocompatibility and water solubility of the PLNPs via a three-step procedure (see the SI).[5] The amino group in PEI on the PLNPs rendered the nanoparticles water-soluble and also enabled conjugation of the PLNPs with Ab-AuNPs to obtain the FRET inhibition probe (PEI-PLNPs/Ab-AuNPs) for AFP detection (see the SI).

To obtain better FRET efficiency, AuNPs were used as the quencher because of their high molar adsorption coefficient and easy surface modification. In addition, the PL emission peak of the PEI-PLNPs was adjusted to ~521 nm, where the Ab-AuNPs gave the maximal absorbance (Figure 1A). For this purpose, the Mg^{2+}/Ca^{2+} molar ratio in the starting materials was adjusted to 1:9 to give maximum overlap between the absorption spectrum of Ab-AuNPs and the PL emission spectrum of the PEI-PLNPs, resulting in the maximum FRET efficiency (Figure 1A and Figure S3B).

The electrostatic interaction between the positively charged $-NH_3^+$ groups of the PEI-PLNPs (ζ potential = +14.25 mV) and the negatively charged antibodies on the surface of the AuNPs (ζ potential = −28.95 mV) in PBS buffer at pH 7.4 should be responsible for the formation of the FRET inhibition probe PEI-PLNPs/Ab-AuNPs (ζ potential = −0.8 mV). Neutralization of the positive charges of the PEI-PLNPs led to no interaction of PEI-PLNPs with Ab-AuNPs (Figure S4A), indicating the electrostatic nature of the interaction between PEI-PLNPs and Ab-AuNPs.[8] Such an electrostatic interaction was also supported by the effect of ionic strength on the PL of PEI-PLNPs/Ab-AuNPs. High ionic strength led to a significant PL recovery of PEI-PLNPs as a result of decreased FRET inhibition by Ab-AuNPs (Figure S4B).

Addition of AFP to PEI-PLNPs/Ab-AuNPs in PBS buffer at pH 7.4 led to an obvious recovery of the PL emission of PEI-PLNPs because of the desorption of Ab-AuNPs from PEI-PLNPs as a result of the competition of AFP with PEI-PLNPs for Ab-AuNPs due to the strong and specific affinity of AFP to the antibody (Figure 2A). A linear relationship between the enhanced PL intensity (ΔP) of the PEI-PLNPs and the concentration of added AFP from 0.8 to 45.0 $\mu g\ L^{-1}$ with a correlation coefficient $R = 0.990$ was observed (Figure 2B). The relative standard deviation for 11 replicate measurements of AFP at 0.8 $\mu g\ L^{-1}$ was 2.3%, and the detection limit (DL) for AFP was 0.41 $\mu g\ L^{-1}$.[9] The DL is comparable to those of most fluorescent and electrochemical sensors for AFP (Table S1 in the SI).[10−17] Moreover, the FRET inhibition probe PEI-PLNPs/Ab-AuNPs exhibits high selectivity to AFP because of the specific affinity of Ab-AuNPs to AFP (Figure 2C).

The PEI-PLNPs/Ab-AuNPs probe was successfully employed to detect AFP in human serum samples. To avoid fluorescent background noise originating from in situ excitation, a solution with a proper amount of the FRET inhibition probe was irradiated for 10 min before detection, eliminating the need for

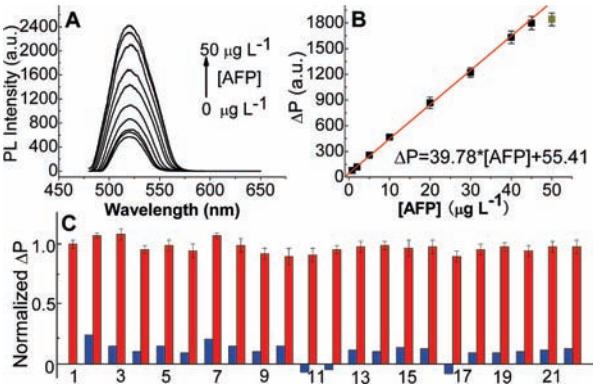

Figure 2. (A) Effect of AFP concentration on the PL emission of PEI-PLNPs/Ab-AuNPs. (B) Plot of enhanced PL intensity (ΔP) against AFP concentration over the linear range 0.8−45.0 $\mu g\ L^{-1}$. (C) PL responses of PEI-PLNPs/Ab-AuNPs to diverse analytes in 10 mM PBS buffer (pH 7.4). Blue bars: (2−8) Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+},Cl^-, and PO_4^{3-} (150.0 mM), respectively; (9−15) human serum albumin (40 g L^{-1}), immunoglobulin G (17 g L^{-1}), transferrin (5 g L^{-1}), glucose, urea, ascobic acid, and citric acid (400 μM), respectively; (16−22) leucine, isoleucine, methionine, phenylalanine, threonine, histidine, and lysine (10.0 mM), respectively. Red bars: [AFP] = 10.0 $\mu g\ L^{-1}$ (1) alone and (2−19) with the corresponding analyte. ΔP denotes the enhanced PL intensity of PEI-PLNPs/Ab-AuNPs in the presence of AFP. The ΔP for [AFP] = 10.0 $\mu g\ L^{-1}$ was set to 1.0 to normalize the PL response.

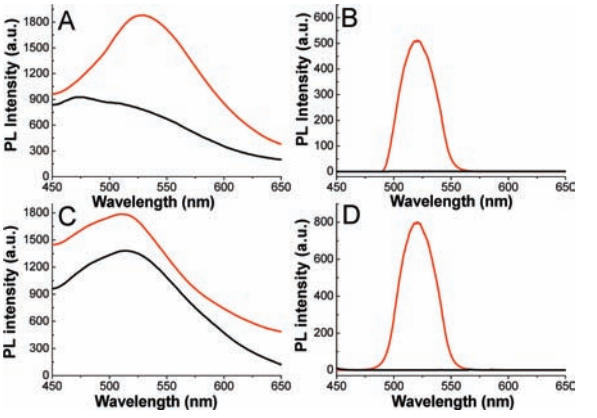

Figure 3. (A, B) PL spectra of a serum sample with detectable AFP in the absence (black line) or presence (red line) of PEI-PLNPs/Ab-AuNPs (3 g L^{-1}) (A) under in situ excitation or (B) upon removal of the excitation source after excitation. (C, D) PL spectra of NIH-3T3 cells in Dulbecco's modified Eagle's medium in the absence (black line) or presence (red line) of PEI-PLNPs/Ab-AuNPs (2 g L^{-1}) and AFP (10 $\mu g\ L^{-1}$) (C) under in situ excitation or (D) upon removal of the excitation source after excitation. A significant PL background from the serum and cellular fluid and asymmetric PL spectra of PEI-PLNPs were observed under in situ excitation, whereas symmetric PL spectra of PEI-PLNPs and no PL background from the serum and cellular fluid were observed after removal of the in situ excitation source.

further excitation during the PL analysis. Because the long-lasting afterglow nature of PLNPs allows detection without external illumination, the autofluorescence and scattering light from biological matrixes encountered under in situ excitation are effectively eliminated (Figure 3 A,B). The analytical results for AFP in six serum samples were in good agreement with those obtained using an enzyme-linked immunosorbent assay (ELISA) (Table S2).

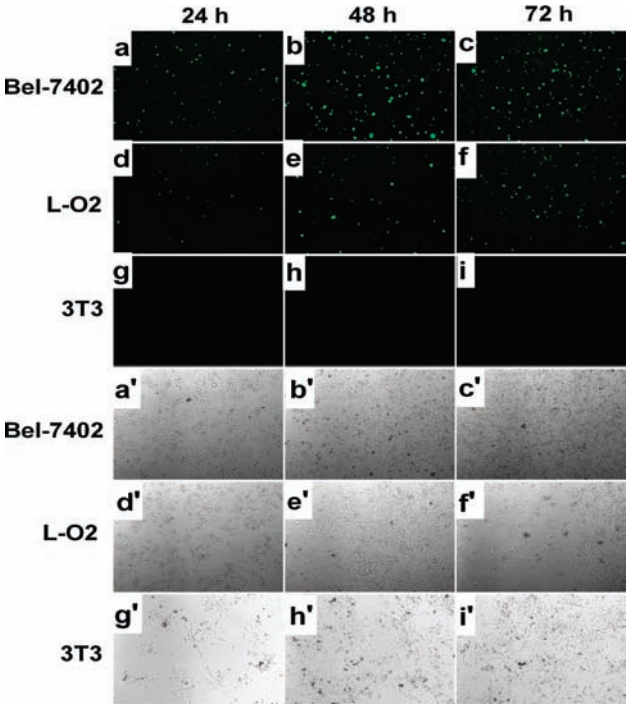

Figure 4. (a−i) Fluorescence images of Bel-7402, L-O2, and 3T3 cells stained with the FRET inhibition probe (PEI-PLNPs/Ab-AuNPs) after the cells had been cultured for 22, 46, and 70 h, respectively and (a′−i′) the corresponding bright-field images.

We also used the PEI-PLNPs/Ab-AuNPs probe to monitor AFP excretion by Bel-7402 cells (a kind of malignant HCC cell with a high growth rate and great ability to excrete AFP)[18] and L-O2 cells (a kind of normal hepatic cells that give a positive result for AFP detection) using the 3T3 cell line as control. Quantitative detection of the AFP excreted during cell growth was carried out after the cells had been cultured for 24, 48, and 72 h, respectively (Figure S5). The PEI-PLNPs/Ab-AuNPs probe was also employed to stain cells that had been cultured for 22, 46, and 70 h, respectively, and the images were observed after incubation of the probe with the cells for 2 h (Figure 4; also see the SI). The PEI-PLNPs were excited with a UV lamp before incubation with Ab-AuNPs. After the incubation, the probe was no longer excited during quantitative detection and imaging to avoid the autofluorescence and scattering light from the biological matrixes (Figure 3 C,D). The results showed a much greater amount of excreted AFP and much faster cell growth for Bel-7402 cells than for L-O2 cells (Figure 4 and Figure S5).

In conclusion, we have prepared water-soluble PEI-PLNPs for use in a FRET inhibition assay for α-fetoprotein excreted during cancer cell growth. The bioconjugates of PEI-PLNPs with Ab-AuNPs provide a sensitive and specific persistent luminescence probe for detection of AFP in biological fluids and for imaging of AFP excreted during cancer cell growth. The probe is stable in biological media (Figure S6) and does not contain toxic heavy metals, thus avoiding the potential toxicity from heavy metals (Figures S6 and S7). Their long-lasting afterglow nature allows detection and imaging without external illumination, thereby eliminating the autofluorescence and scattering light from biological matrixes encountered under in situ excitation. Although the present work is a cellular assay and the studies here are for microscopy rather than in vivo imaging, the developed assay has potential for in vivo applications.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details and additional figures and tables mentioned in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
xpyan@nankai.edu.cn

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(9) Here the DL is defined as the concentration of AFP corresponding to the sum of 3 times the standard deviation of ΔP for 11 replicate detections of AFP at 0.8 μg L^{-1} and the ΔP caused by 15 mM NaCl solution (i.e., 10× dilution of a physiological salt solution). Thus, the DL was calculated by dividing the sum of 3 times the standard deviation of ΔP for 11 replicate detections of AFP at 0.8 μg L^{-1} and the ΔP caused by 15 mM NaCl solution by the slope of the calibration function.
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