Electronic Supplementary Information

Luminescent Europium Nanoparticles with a Wide Excitation Range from UV to Visible Light for Biolabeling and Time-Gated Luminescence Bioimaging

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Experimental Section

Materials and Physical Measurements.

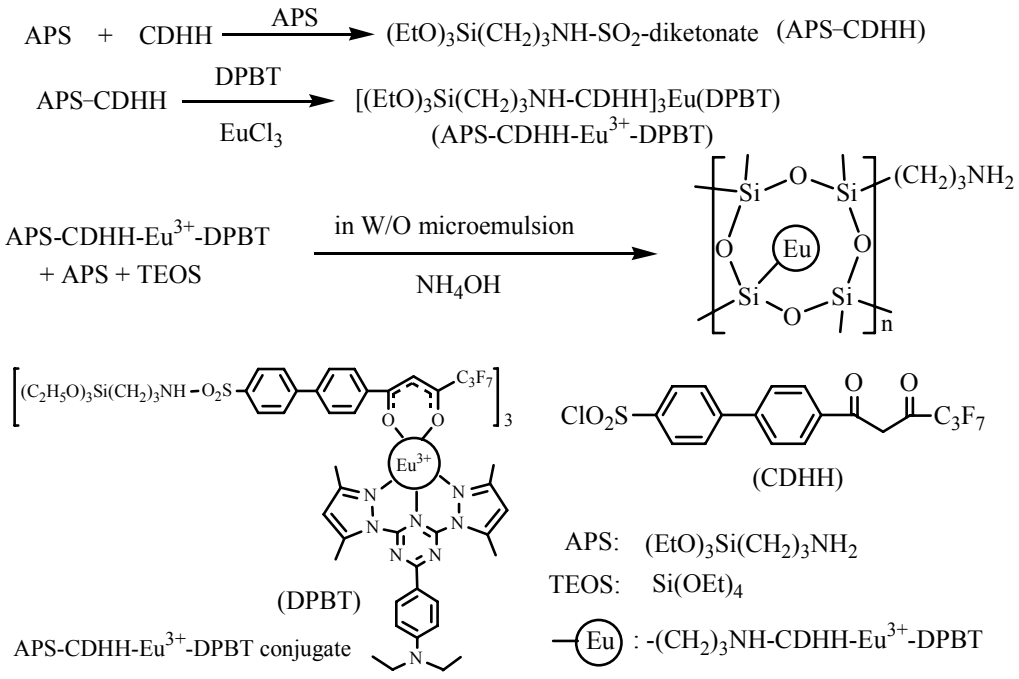
5-(4”-Chlorosulfo-1’,1”-diphenyl-4’-yl)-1,1,1,2,2,3,3-heptafluoro-4,6-hexanedione (CDHH) and 2-(N,N-diethylanilin-4-yl)-4,6-bis(3,5-dimethylpyrazol-1-yl)-1,3,5-triazine (BPDT) were synthesized by using previous methods,[1,2] respectively. 3-Aminopropyl(triethoxyl)silane (APS), tetraethyl orthosilicate (TEOS), and dioctyl sulfosuccinate (AOT) were purchased from Acros Organics. Streptavidin (SA) was purchased from Chemicon International Inc. Mouse monoclonal and goat polyclonal anti-human prostate-specific antigen (PSA) antibodies and rabbit anti-mouse IgG antibody were purchased from OEM Concepts Co. Biotinylated goat anti-human PSA and rabbit anti-mouse antibodies were prepared and used according to a previous method.[3] *Giardia lamblia* and its mouse monoclonal antibody were purchased from Biotech Frontiers Pty. Ltd. The water sample of marine green alga *Platymonas subcordiformis* was provided by marine bioproducts engineering group of Dalian Institute of Chemical Physics, Chinese Academy of Sciences. Natural seawater was collected from China Yellow Sea near Dalian city. The standard solutions of human PSA were prepared by diluting human PSA antigen (Biogenesis Ltd.) with 0.05 M Tris-HCl buffer of pH 7.8 containing 5% bovine serum albumin (BSA), 0.9% NaCl, and 0.1% NaN_3. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without

further purification.

The shape and size of the nanoparticles were characterized by using a JEOL JEM-2000EX transmission electron microscope (TEM). Luminescence spectra and emission lifetimes were measured on a Perkin-Elmer LS 50B spectrofluorometer. Luminescence quantum yield (ϕ) of the nanoparticles was measured by using a previous method.[4] The time-resolved fluoroimmunoassay (TR-FIA) of human PSA was carried out with a FluoroNunc 96-well microtiter plate as solid-phase carrier and measured on a Perkin-Elmer Victor 1420 multilabel counter at the following conditions: excitation wavelength, 340 nm; emission wavelength, 615 nm; delay time, 0.2 ms; and window time (counting time), 0.4 ms. All normal luminescence imaging and time-gated luminescence imaging measurements were carried out on a laboratory-use luminescence microscope.[5] The microscope equipped with a 100 W mercury lamp, a UV-2A filters (excitation filter, 330-380 nm; dichroic mirror, 400 nm; emission filter, > 420 nm), a V-2A filters (excitation filter, 380-420 nm; dichroic mirror, 430 nm; emission filter, > 450 nm) and a color CCD camera system were used for the normal luminescence imaging measurement with an exposure time of 500 ms. The microscope equipped with a 30 W xenon flashlamp, UV-2A and V-2A filters, and a time-gated digital black-and-white CCD camera system were used for the time-gated luminescence imaging measurement at the conditions of delay time, 100 μs; gate time, 1 ms; lamp pulse width, 6 μs; and exposure time, 120 s.

Preparation of the Nanoparticles.

The preparation principle of the nanoparticles is shown in Scheme S1.



Scheme S1. Preparation principle of the nanoparticles.

Before the preparation of nanoparticle, the APS-CDHH-Eu^{3+}-BPDT conjugate was synthesized mainly according to a previous method.[4] To 175 μL (0.75 mM) of APS was added a solution of CDHH (73.62 mg, 0.15 mM) in 4 ml of dry tetrahydrofuran (THF) with stirring. After the mixture was stirred at room temperature for 3 h, DPBT (20.83 mg, 0.05 mM) dissolved in 15 mL of dry THF and $EuCl_3 \cdot 6H_2O$ (18.35 mg, 0.05 mM) were added. The mixture was further stirred for 24 h at room temperature to give a yellow solution with very bright red luminescence in daylight. After evaporation, the residue was re-dissolved in a small amount of diethyl ether. Addition of n-hexane to the solution led to the precipitation of APS-CDHH-Eu^{3+}-BPDT conjugate as a yellow powder. The procedure of nanoparticle preparation was as follows. To a W/O microemulsion prepared by mixing 0.45 g of AOT, 8.5 mL of heptane and 90 μL of water was added 16 mg of the above conjugate dissolved in 1.5 mL of toluene. After the solution was stirred for 30 min at room temperature, 100 μL of TEOS and 10 μL of APS were added. The copolymerization reaction was initiated by adding 100 μL of concentrated aqueous ammonia, and the reaction was allowed to continue for 24 h at room temperature. The yellow solid nanoparticles were isolated from the microemulsion by adding 0.35 mL of ethanol, centrifuging, and washing with water and 0.1 M phosphate buffer of pH 7.1 several times to remove surfactant and unreacted materials.

Preparation of the Nanoparticle-Labeled SA.

The nanoparticle-labeled SA was prepared according to a previous method.[4] To 1.2 mL of 0.1 M phosphate buffer of pH 7.1 containing 5 mg of BSA were added 1.0 mg of the nanoparticles and 0.3 mL of 1% glutaraldehyde. After stirring for 22 h at room temperature, 1.0 mg of $NaBH_4$ was added, and the solution was incubated for 2 h at room temperature. The nanoparticles were centrifuged and washed with the phosphate buffer and then added to 0.9 mL of the phosphate buffer containing 0.4 mg of SA. After 0.2 mL of 1% glutaraldehyde was added and the reaction mixture was stirred for 22 h at room temperature, 1.0 mg of $NaBH_4$ was added and the solution was incubated for 2 h at room temperature. The nanoparticle-labeled SA was centrifuged and washed three times with the phosphate buffer, and further purified by gel filtration chromatography on a Sephadex G-50 column with 0.05 M NH_4HCO_3 of pH 8.0 as the eluent (2.9 mL of the nanoparticle-labeled SA solution was obtained). After 2.9 mg of NaN_3 and 5.8 mg of BSA were added, the nanoparticle-labeled SA solution was stored at 4 °C and diluted with 0.05 M Tris-HCl buffer of pH 7.8 containing 0.2% BSA, 0.1% NaN_3, and 0.9% NaCl before use.

The luminescence image of the nanoparticle-labeled SA was measured to observe the aggregation of the nanoparticles.

TR-FIA of Human PSA.

After anti-human PSA monoclonal antibody (diluted to 10 μg /mL with 0.1 M carbonate buffer of pH 9.6) was coated on the wells (50 μL/well) of a 96-well microtiter plate by physical adsorption,[6] 50 μL of human PSA standard solutions were added to the wells. The plate was incubated at 37 °C for 1 h, and washed twice with 0.05 M Tris-HCl buffer of pH 7.8 containing 0.05% Tween 20 and once with 0.05 M Tris-HCl buffer of pH 7.8. Then the biotinylated goat anti-human PSA antibody (~1.1 μg/mL, 50 μL/well) was added to each well, and the plate was incubated at 37 °C for 1 h. After washing, the nanoparticle-labeled SA (~2.0 μg/mL, 50 μL/well) was added to each well, and the plate was incubated at 37 °C for 1 h. The plate was washed four times with 0.05 M Tris-HCl buffer of pH 7.8 containing 0.05% Tween 20, and subjected to solid-phase time-gated luminescence measurement on Perkin-Elmer Victor 1420 multilabel counter.

Luminescence Imaging of *Giardia lamblia.*

Five μL of *Giardia lamblia* solution (10^6 cysts/mL) was mixed with 20 μL of anti-*Giardia* antibody (40 μg/mL), 20 μL of biotinylated rabbit anti-mouse antibody (40 μg/mL) and 8 μL of the nanoparticle-labeled SA solution (~30 μg/mL) in a tube. After incubation for 24 h at room temperature, the cysts were separated by centrifugation at 500 rpm and washed with distilled water three times to remove the unreacted nanoparticle-SA conjugate. The cysts were mixed with the environmental water samples, and then spotted on a glass slide for luminescence microscopy imaging detection. To confirm the non-specific binding of the nanoparticles on *Giardia* cysts, a control experiment in the absence of anti-*Giardia* antibody was also carried out.

RESULTS

Luminescence Spectra of CDHH-Eu^{3+}-DPBT Complex in Different Solvents.

Figure S1 shows the time-gated excitation and emission spectra of the CDHH-Eu^{3+}-DPBT complex, $(CDHH)_3Eu(DPBT)$, in toluene and in ethanol, respectively. In toluene, the complex shows three excitation peaks at 296, 343 and 400 nm. In ethanol, the complex shows two excitation peaks at 289 and 338 nm. These results clearly indicate that the longer excitation wavelengths caused by the singlet energy transfer pathway of visible-light-sensitized β-diketonate-Eu^{3+}-DPBT complexes in toluene[2] could be prevented in ethanol, a stronger coordinating solvent, due to the interaction of center Eu^{3+} ion with ethanol molecules.

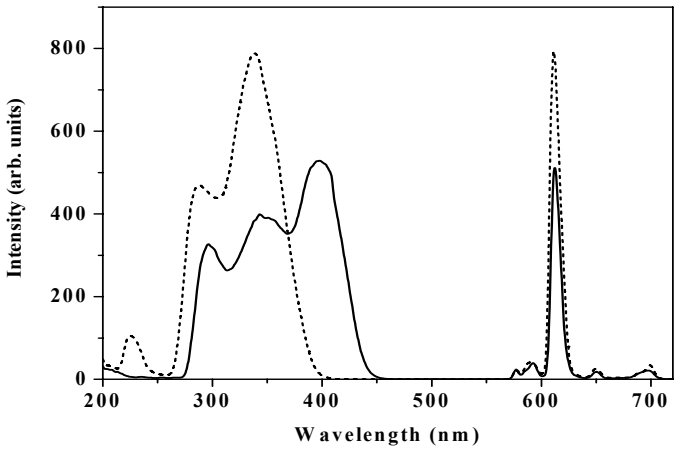


Figure S1. Time-gated excitation and emission spectra of the $(CDHH)_3Eu(DPBT)$ complex in toluene (1.0×10^{-5} mol/L, solid line) and in ethanol (1.0×10^{-6} mol/L, dashed line).

Luminescence Imaging of the Nanoparticle-Labeled SA.

Figure S2 shows the luminescence image (excited with 330-380 nm) of the nanoparticle-labeled SA in aqueous buffer. It can be observed that the nanoparticles in the solution are relatively well-dispersed with some aggregations. However, because the aggregation of the nanoparticles is not serious, it has not obvious effect on cell staining and time-gated luminescence bioimaging applications.

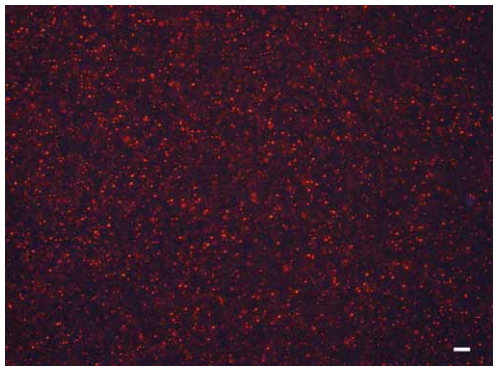


Figure S2. Luminescence image (excited with 330-380 nm) of the nanoparticle-labeled SA in 0.05 M NH_4HCO_3 of pH 8.0. Scale bars, 10 μm.

TR-FIA of Human PSA.

The PSA in human serum as an important tumor marker has been widely accepted and used in the diagnosis of prostatic cancer and breast cancer,[7] and had been measured with various immunoassays including enzyme immunoassay,[8] chemiluminescence immunoassay[9] and TR-FIA.[3,10] Therefore, the immunoassay of PSA was used to confirm the reactivity of the nanoparticle-labeled SA with biotinylated antibody and to evaluate the usefulness of the

nanoparticle-labeled SA for TR-FIA. The calibration curve of TR-FIA for human PSA is shown in Figure S3. The straight line in the PSA concentration range of 0.1-10 ng/mL can be expressed as log(signal) = 0.732 log[PSA] + 4.47 (r = 0.999). The detection limit, calculated with the concentration corresponding to three standard deviations of background signal, is 0.1 ng/mL, which basically amounts to the detection limit of commercially available PSA assay methods. This result indicates that the new nanoparticles can be used as a label for highly sensitive TR-FIA.

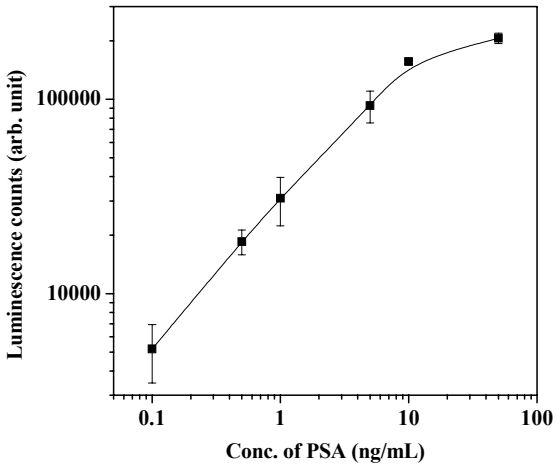


Figure S3. Calibration curve of TR-FIA by using the nanoparticle-labeled SA for human PSA.

Luminescence Imaging of *Giardia.*

Figure S4 shows the bright-field and time-gated luminescence (excited with 330-380 nm and 380-420 nm, respectively) images of *Giardia lamblia* stained by mouse anti-*Giardia* antibody, biotinylated rabbit anti-mouse antibody and nanoparticle-labeled SA. The clear images detected by using both 330-380 nm and 380-420 nm excitation filters demonstrate that the nanoparticle-based luminescence label prepared in this work can be excited by both UV and visible lights for time-gated luminescence bioimaging application.

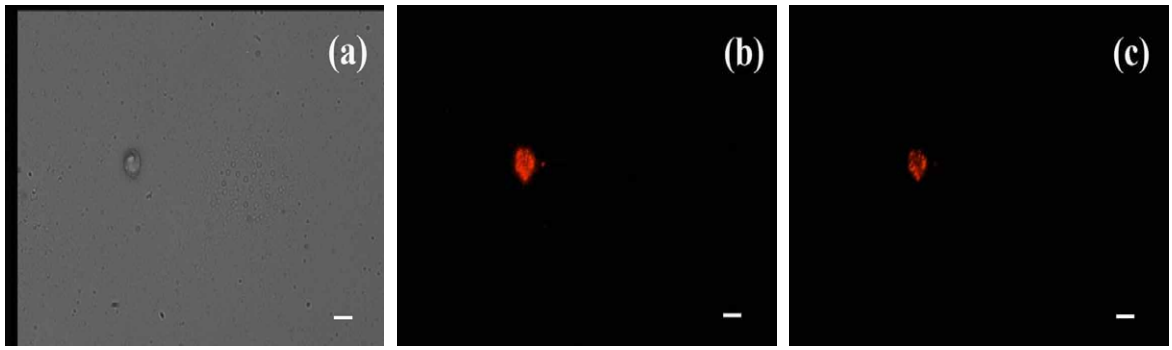


Figure S4. Bright-field (a) and time-gated luminescence (b, c, excited with 330-380 nm and 380-420 nm, respectively) images of *Giardia lamblia* stained by mouse anti-*Giardia* antibody, biotinylated rabbit anti-mouse antibody and nanoparticle-labeled SA. Scale bars, 10 μm. The

time-gated luminescence images are shown in pseudo-color (wavelength of 615 nm) treated by a SimplePCI software.[5]

The control experiment in the absence of the anti-*Giardia* antibody by luminescence imaging method was conducted to explore the non-specific binding of the nanoparticles on *Giardia* cysts. The result is shown in Figure S5. The results indicate that the non-specific binding of the nanoparticles on *Giardia* cysts does not occur in the absence of the anti-*Giardia* antibody.

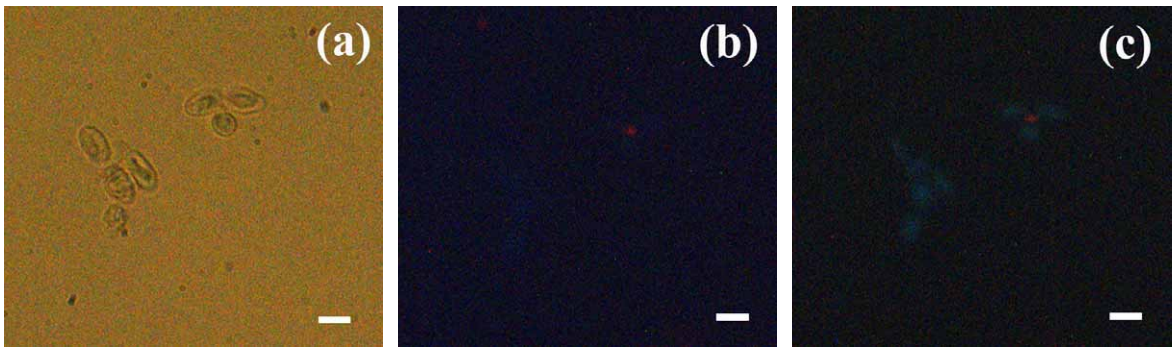


Figure S5. Bright-field (a) and luminescence (b, c, excited with 330-380 nm and 380-420 nm, respectively) images of *Giardia lamblia* stained by biotinylated rabbit anti-mouse antibody and nanoparticle-labeled SA without anti-*Giardia* antibody. Scale bars, 10 μm.

References

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