Electronic supplementary information

Visible-light-sensitized highly luminescent europium nanoparticles: preparation and application for time-gated luminescence bioimaging

Jing Wu, Zhiqiang Ye, Guilan Wang, Dayong Jin, Jingli Yuan, Yafeng Guan, James Piper

1. Experiments

Materials. Mouse monoclonal and goat polyclonal anti-human prostate-specific antigen (PSA) antibodies were purchased from OEM Concepts Co. Biotinylated goat anti-human PSA was prepared and used according to a previous method.[1] 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.

Instruments. 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 with 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 bright-field imaging, normal luminescence imaging and time-gated luminescence imaging measurements were carried out on a laboratory-use luminescence microscope.[2]

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,[3] 45 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, 45 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, 45 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.

Control Experiment of Luminescence Imaging of *Giardia lamblia.* To confirm the non-specific binding of the nanoparticles on *Giardia* cysts, a control experiment in the absence of anti-*Giardia*

antibody was carried out. Five μL of *Giardia lamblia* solution (2×10^6 cysts/mL) was mixed with 16 μL of biotinylated rabbit anti-mouse antibody (50 μg/mL) and 6 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 diluted with distilled water, and then spotted on a glass slide for luminescence microscopy imaging detection.

2. Results

Effect of pH on the luminescence intensity of the nanoparticles. The effect of pH on the luminescence intensity of the nanoparticles was measured in 0.05 M Tris-HCl buffers with different pHs (from 2 to 11). As shown in Figure S1, the effect of pH on luminescence intensity is small in the range of pH 6.0 to 11.0 (intensity change less than 10%), which indicates that the luminescence of the nanoparticles is stable in physiological pH range.

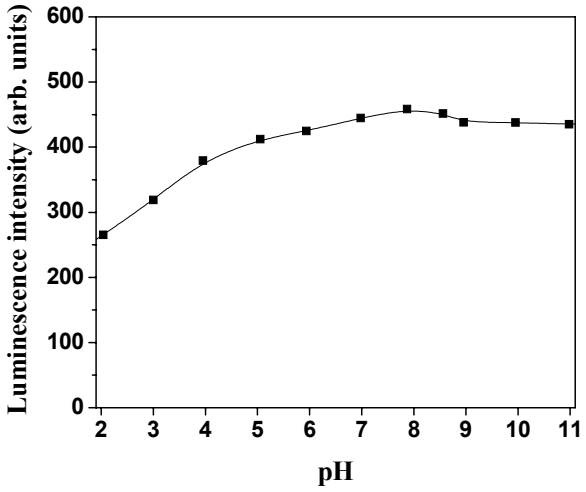

Figure S1. Effect of pH on the luminescence intensity of the nanoparticles.

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,[4,5] and had been measured with various immunoassays including enzyme immunoassay,[6] chemiluminescence immunoassay[7] and TR-FIA.[1,8] Herein, the immunoassay of PSA was used to confirm the reactivity of the nanoparticle-labeled SA with biotinylated 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 S2. The straight line in the PSA concentration range of 0.01-5 ng/mL can be expressed as log(singal) = 0.735 log[PSA] + 4.843 (r =

0.996). The detection limit, calculated with the concentration corresponding to three standard deviations of background signal, is 29.8 pg/mL, which is obviously higher than the detection limits of commercially available PSA assay methods. This result indicates that the new nanoparticles can be used as a label for highly sensitive TR-FIA.

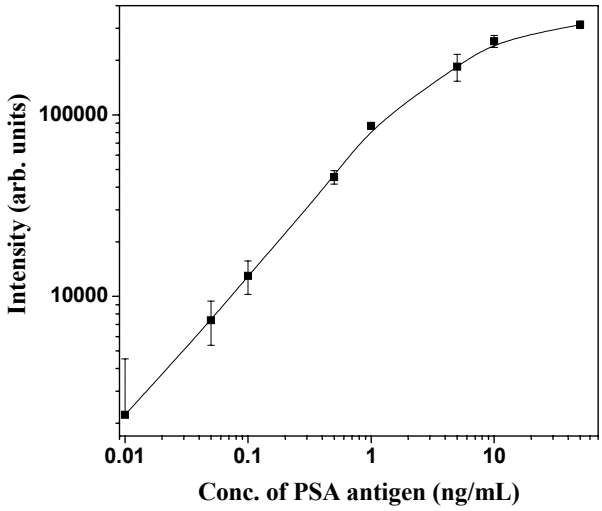

Figure S2. Calibration curve of TR-FIA for human PSA by using the nanoparticle-labeled SA.

Control experiment of Luminescence Imaging of *Giardia lamblia.* 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 results shown in Figure S3 indicate that the non-specific binding of the nanoparticles on *Giardia* cysts does not occur in the absence of the anti-*Giardia* antibody.

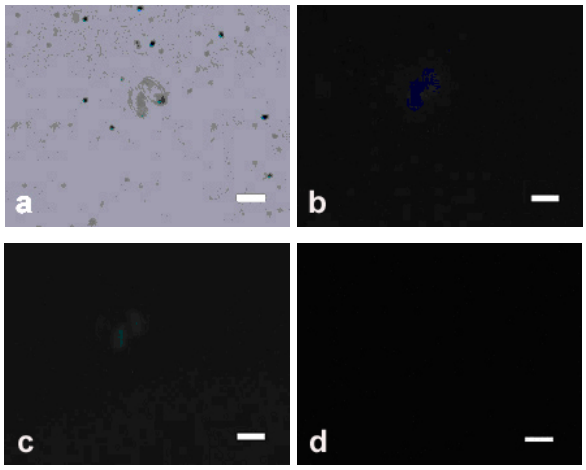

Figure S3. Bright-field (a), luminescence (b, c, excited with 330-380 nm and 380-420 nm, respectively) and time-gated luminescence (d, excited with 330-380 nm) images of *Giardia lamblia* stained by

biotinylated rabbit anti-mouse antibody and nanoparticle-labeled SA without anti-*Giardia* antibody. Scale bars, 10 μm. 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 was used for the normal luminescence imaging measurement with an exposure time of 6 s. The microscope equipped with a 30 W xenon flashlamp, a UV-2A filters, and a time-gated digital black-and-white CCD camera system was used for the time-gated luminescence imaging measurement with the conditions of delay time, 100 μs; gate time, 1 ms; lamp pulse width, 6 μs; and exposure time, 240 s. The microscope equipped with a 30 W halogen lamp, and the color CCD was used for the bright-field imaging measurement with an exposure time of 0.3 s.

References

1. Z. Q. Ye, M. Q. Tan, G. L. Wang and J. L. Yuan, *Anal. Chem*., 2004, **76**, 513-518.
2. B. Song, G. L. Wang, M. Q. Tan and J. L. Yuan, *J. Am. Chem. Soc.*, 2006, **128**, 13442-13450.
3. K. Matsumoto, J. L. Yuan, G. L. Wang and H. Kimura, *Anal. Biochem*., 1999, **276**, 81-87.
4. D. A. Armbruster, *Clin. Chem*., 1993, **39**, 181-195.
5. R. A. Ferguson, H. Yu, M. Kalyvas, S. Zammit and E. P. Diamandis, *Clin. Chem*., 1996, **42**, 675-684.
6. R. L. Vessella, J. Noteboom and P. H. Lange, *Clin. Chem*., 1992, **38**, 2044-2054.
7. R. F. Dudley, *Lab. Med*., 1990, **21**, 216-222.
8. Q.-P. Qin, T. Lövgren and K. Pettersson, *Anal. Chem*., 2001, **73**, 1521-1529.