Electronic Supplementary Information

Bioconjugated Persistent Luminescence Nanoparticles for Föster Resonance Energy Transfer Immunoassay of Prostate Specific Antigen in Serum and Cell Extract without in Situ Excitation

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EXPERIMENTAL SECTION

Chemicals and reagents

All chemicals used were at least analytical grade. Ultrapure water (18.2 MΩ cm) obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, MO) was used throughout. N-hydroxysuccinimide (NHS) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) were from Aldrich (Steinheim, Germany). 3-aminopropyltriethoxysilane (APTES) was from Aladdin (Shanghai, China). RhB, Na_2HPO_4, $Zn(NO_3)_2·6H_2O$, KH_2PO_4, $CaCl_2$, $Mg(NO_3)_2·6H_2O$, ethyl orthosilicate (TEOS), dimethylformamide (DMF), diglycolic anhydride and dysprosium oxide were from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). $Eu(NO_3)_3·5H_2O$ and Dy_2O_3 were from Fengyue Chemical Reagent Institue (Tianjin, China). All the other amino acids, reduced GSH and human serum albumin (HSA) were from Newprobe Biotechnology Co. Ltd. (Beijing, China). PSA and mouse monoclonal PSA antibodies (PS6 and 8A6) were from Jingtian Biotech Co. Ltd. (Shanghai, China). PC-3 human prostate cancer cells and RWPE-1 human normal prostate epithelial cells were purchased from the Committee on Type Culture Collection of the Chinese Academy of Sciences.

Instrumentation

The morphology of the PLNP was characterized by transmission S2 electron microscopy (TEM) on a JEOL-100CX-Ⅱ (JEOL, Japan) microscope operating at a 200 kV accelerating voltage. The samples for TEM were obtained by drying sample droplets from water dispersion onto a 300-mesh Cu grid coated with a carbon film, which was then allowed to dry prior to imaging. The persistent luminescence (PL) spectra of PLNP and RhB were recorded on an F-4500 spectrofluorometer (Hitachi, Japan) equipped with a plotter unit and a quartz cell (1 cm × 1 cm) with a PMT votage of

700 V without in situ excitation. Fourier transform infrared (FT-IR) spectra (4000-400cm^{-1}) in KBr were recorded on a Magna-560 spectrometer (Nicolet, Madison, WI).

Synthesis of Carboxyl Modified PLNP.

The PLNP was synthesized according to our previous work with a little modification. Briefly, Dy_2O_3 powder was dissolved in concentrated nitric acid under vigorously heating to get $Dy(NO_3)_3$ (0.1 M). $Zn(NO_3)_2 \cdot 6H_2O$, $CaCl_2$, $Mg(NO_3)_2 \cdot 6H_2O$, $Eu(NO_3)_3 \cdot 5H_2O$ were dissolved in ultrapure water to get the final concentration of 0.1 M respectively. 4.5 mL $CaCl_2$, 0.5 mL $Mg(NO_3)_2$, 2.5 mL $Zn(NO_3)_2$, 0.05 mL $Eu(NO_3)_3$ were mixed and stirred at room temperature. After 0.04 mL $Dy(NO_3)_3$ added, the solution was acidified at pH 2 with concentrate nitric acid. TEOS was then added rapidly, and the solution was stirred at room temperature until the solution became transparently. The solution was then refluxed until the sol-to-gel transition occurred. To obtain opaque gel, the wet gel was dried in an oven at 110 ^{o}C. The gel was then calcined in microwave oven at 950 w for 10 min in a reducing atmosphere of active carbon to crystallize and form the luminescence center.

For carboxyl modification, 300 mg of PLNP with 20 mL of ultrapure water, 50 μL of 1.5 M NaOH, 10 μL of APTES and 250 μL of TEOS were mixed under stirring. The suspension was then kept stirring and refluxed for 8 h. The resultant mixture was centrifuged and washed to obtain APTES coated PLNP. 150 mg of APTES coated PLNP were dispersed in DMF, and then diglycolic anhydride (42.7 mg) was added. The suspension was stirred overnight at room temperature and the mixture was centrifuged, washed, and dried to get the carboxyl functionalized PLNP.

Bioconjugation of the Mouse Monoclonal PSA Antibody PS6 and PLNP.

A carbodiimide method was used to bioconjugate the anti-PSA monoclonal antibody (PS6) onto PLNP. Briefly, 10 mg of PLNP were dispersed into 5 mL of phosphate buffer saline (PBS) buffer (0.1 M, pH 7.4). 15 mg of NHS and 15 mg of EDC were quickly added to the above suspension, and allowed to react for 30 min at room temperature with stirring. The mixture was centrifuged (6000 rpm, 5 min) and the precipitant was redispersed in 5 mL of PBS buffer (0.1 M, pH 7.4). Then, 5 mg of PS6 in 0.5 mL PBS was added at room temperature for another 4 h reaction under soft stirring. The resultant PLNP-PS6 was centrifuged (4 ^{o}C, 6000 rpm, 5 min), washed with PBS buffer for three times, freeze-dried, and kept at 4 ^{o}C.

Preparation of RhB Labeled Mouse Monoclonal PSA Antibody (RhB-8A6).

10 mg of RhB was dissolved in 5.0 mL of PBS buffer (0.1 M, pH 7.4) and reacted with 10 mg of NHS and 10 mg EDC for 30 min to activate the carboxyl groups. 4 mg of mouse monoclonal PSA antibody 8A6 was then added into the above solution and incubated at 37 ^{o}C for 1 h. After incubation, 20 mg of HSA was added to seal the unreacted carboxyl groups of RhB. Finally, the mixture was centrifuged (4 ^{o}C, 12000 rpm, 15 min) and washed with PBS buffer for 3 times to obtain RhB-8A6. The RhB-8A6 was kept at 4 ^{o}C in 2 mL of PBS buffer (0.1 M, pH 7.4). The final concentration of RhB-8A6 was 6 g L^{-1}.

Preparation of Biological Samples.

The serum samples of the patients with prostate cancer were obtained from Tianjin medical university cancer institute (Tianjin, China). PC-3 human prostate cancer cells and RWPE-1 human normal prostate epithelial cells were cultured for 24 h, washed twice with PBS buffer, and mixed with 2 mL of PBS buffer buffer (0.1 M, pH 7.4). The cell suspension was transferred to a 4-mL

centrifuge tube. The disrupted cell suspension was centrifuged (1200 rpm) for 10 min, and the supernatant was collected. The standard solutions of PSA for calibration were prepared by dissolving PSA in 0.01 M PBS buffer solution (pH 7.4, containing 1% BSA) and stored at 4 ^{o}C.

Procedures for Determination of PSA in Biological Samples.

In a typical test, the PLNP-PS6 was pre-excited with a UV-lamp (5 w, 360 nm) for 20 min to get the afterglow photoluminescence. To confirm the efficiency of the FRET immunoassay, PLNP-PS6 (4.65 mg) and diluted RhB-8A6 solution (4.5 g L^{-1}, 1 mL) were mixed in a 5.0-mL calibrated test tube with PBS buffer (0.1 M, pH 7.4) and stirred for 10 min at room temperature. Then, 1 mL of the biological fluid sample or PSA standard solution was added. The mixture was diluted to volume with PBS buffer, set for 30 min, and measured with an F-4500 spectrofluorometer at an emission wavelength range from 475 nm to 700 nm with an emission slit of 20 nm and a PMT voltage of 700 V without in situ excitation.

REFERENCES

1 B.-Y. Wu, H.-F. Wang, J.-T. Chen and X.-P. Yan, *J. Am. Chem. Soc.* 2011, **133**, 686.

Temperature (°C):	25.1		**Duration Used (s):**	70
Count Rate (kcps):	196.1		**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette		**Attenuator:**	7

			Size (r.nm):	**% Intensity**	**Width (r.nm):**
Z-Average (r.nm):	155.5	**Peak 1:**	151.5	100.0	50.98
PdI:	0.356	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.868	**Peak 3:**	0.000	0.0	0.000
Result quality :	Good				

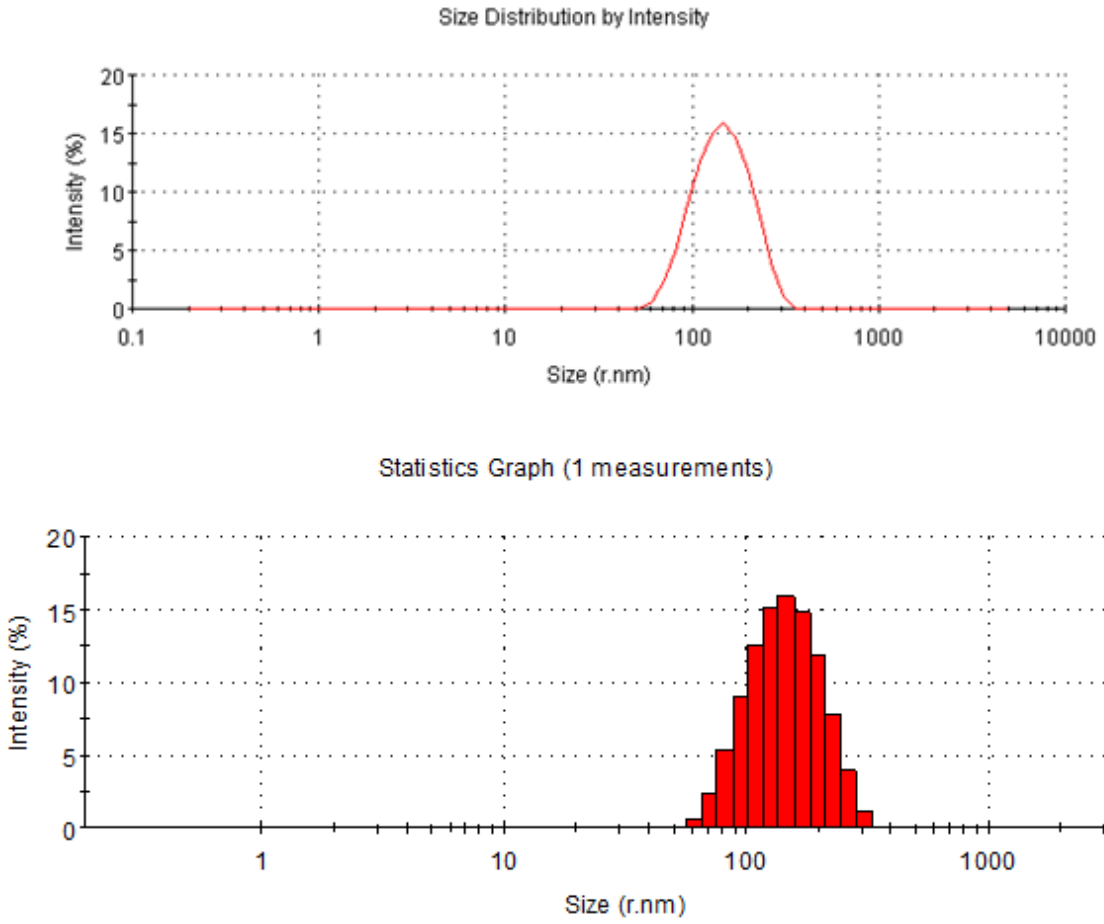

Fig. S1 Particle size distribution of PLNP-PS6 in PBS (0.1M, pH 7.4, 25 **°C**)

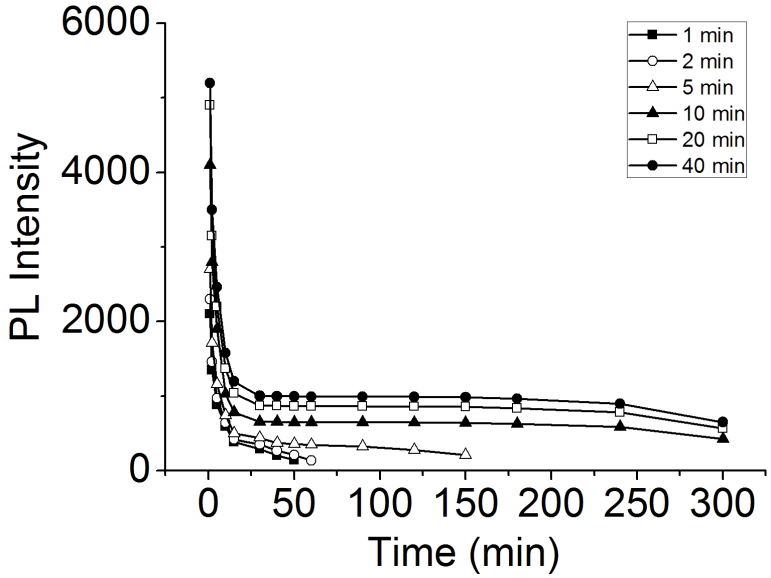

Fig. S2 Time-dependent PL intensity of 2.5 g L^{-1} COOH-PLNP in PBS buffer (0.1 M, pH 7.4) without further excitation after direct exposure under a 360 nm UV lamp under different irradiation time from 1 min to 40 min.

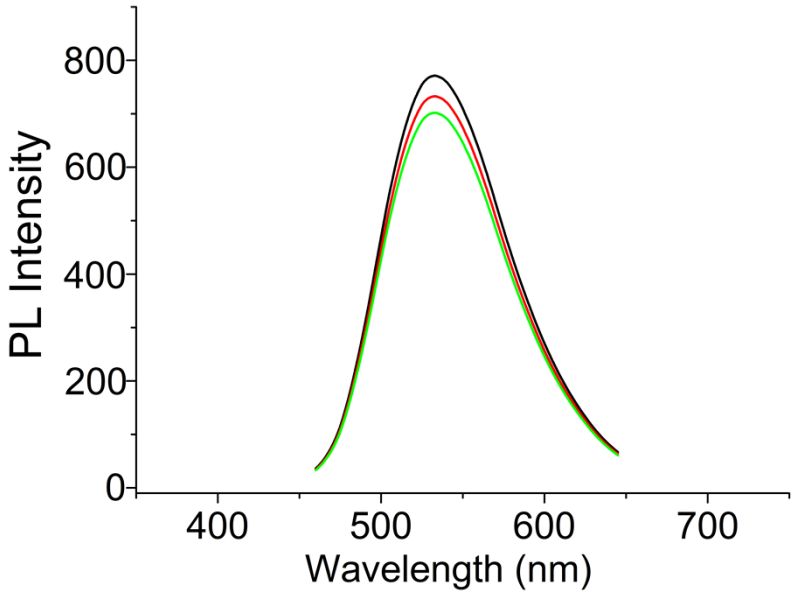

Fig. S3 The stability of the PL intensity of 4.65 g $L^{-1}$of PLNP-PS6 in different chemical environment after incubation with ultrapure water (black curve), PBS (0.1 M pH 7.4, red curve) and serum (green curve).