Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Nanoparticles Based on Quantum Dots and a Luminol Derivative: Implications for *in vivo* Imaging of Hydrogen Peroxide by Chemiluminescence Resonance Energy Transfer

Eun Sook Lee,[a,b] V. G. Deepagan,[c] Dong Gil You,[d] Jueun Jeon,[d] Gi-Ra Yi,[d] Jung Young Lee,[d] Doo Sung Lee,[d] Yung Doug Suh,[*,b,d] and Jae Hyung Park[*,a,b,d]

[a] Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Suwon 440-746, Republic of Korea

[b] Research Center for Convergence Nanobiotechnology (RC2NT), Korea Research Institute of Chemical Technology (KRICT), Daejeon 305-600, Republic of Korea

[c] Department of Polymer Science and Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea

[d] School of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Corresponding Authors

* (J.H.P.) E-mail: jhpark1@skku.edu

* (Y.D.S.) E-mail: ydsuh@krict.re.kr

S1. Experimental details

Materials. Carboxyl quantum dot (QD) was purchased from Invitrogen Life technologies (Carlsbad, CA, USA). Commercial QDs are composed of ZnS-CdSe-CdTe core-shell and coated using amphiphilic polymer which has –Coo^- surface group with the results that QDs are well dispersed in aqueous solutions. Methoxy polyethylene glycol amine (PEG, MW = 5 kDa), *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarboiimde hydrochloride (EDC), *N*-hydroxysuccimide (NHS) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). 8-Amino-5-chloro-7-phenylpyrido [3,4-d] pyridazine-1,4-(2H,3H) dione sodium salt (L012) was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Water used for all the experiments was purified using the AquaMax-Ultra water purification system (Yonglin Co., Anyang, Korea). All other chemicals were of analytical grade and used without further purification.

Preparation and characterization of nanoparticles. HNPs were synthesized by simple EDC chemistry as follows. QD (0.2 nmol) was mixed with EDC (65 µl, 1 mg/ml) and NHS (55 µl, 1 mg/ml). L012 (100 µl, 1 mg/ml) in sodium borate buffer (pH 8.0) was added dropwise to the solution. After vigorous stirring for 12 h at room temperature under the dark condition, EDC (13 µl, 1 mg/ml) and NHS (11 µl, 1 mg/ml) were additionally added. To the resulting solution, PEG (22 µl, 1 mg/ml) in borate buffer (pH 8.0) was added slowly and the reaction was allowed to proceed for 12 h. Thereafter, the products were purified three times using the centrifugal filter (Amicon ultra-15 centrifugal filter units, MWCO = 10kDa, Merck Millipore, Germany) against borate buffer. PEG-QD was also prepared by EDC chemistry, in which the experimental

procedure was the same as that of HNPs except the L012 coupling step. The products were stored at 4 ℃, prior to use. The particle size was measured by using the zetasizer (ZS90, Malvern). The chemical structure of the nanoparticles was characterized using the Fourier transform infrared spectroscopy (FT-IR) and the elemental analyzer (EA). The photoluminescence spectra was monitored using fluorometer.

***In vitro* chemiluminescence (CL) imaging.** *In vitro* CL imaging was performed using the IVIS Lumina series III Pre-clinical *In Vivo* imaging system (Caliper Life Science, Hopkinton, MA, USA). An optical imaging in a 96-well plate was set up as follows: L012 (100 µl), PEG-QD (100 µl) or HNPs (100 µl) in DPBS was added to each well. The concentration of L012 was adapted to the amount of L012 in HNPs according to elemental analysis results, and the concentration of PEG-QD was set according to the amount of quantum dots in HNPs. Then, 100 µl of hydrogen peroxide was added and the images were obtained with auto-exposure time and bioluminescence mode that blocked excitation light. All luminescence images from each well were quantified using embedded software. Data are presented as the mean luminescence signals ± SD (Standard deviation) (n = 3). The CL images of L012, PEG-QD and HNPs were obtained with the open emission filter and with a 790 nm wavelength emission filter after addition of 0.5 µM hydrogen peroxide. The exposure time was 15-sec with open filter and 60-sec with 790 nm filter.

In vivo CL imaging. All animal experiments were performed in compliance with the relevant laws and institutional guidelines of Sungkyunkwan University, and the institutional committees approved the experiments.

In vivo CL imaging was performed using IVIS Lumina series III. L012 (100 µl*)*, PEG-QD (100 µl), and HNPs (100 µl) in Dulbecco's phosphate buffered saline (DPBS) were injected to the animal model. Auto-exposure time in IVIS Lumina was 60-sec and images were taken 3 minutes after local administration of the sample with 790 nm emission filter. The luminescence images from each animal model were quantified using embedded software. Data are shown as the mean luminescence signals ± SD (*n* = 3). PC3 human prostate cancer cells, obtained from the American Type Culture Collection (Rockville, MD, USA), were used to prepare the tumor model. The athymic mice (5 weeks old, Orient Bio Inc., Korea) were inoculated with a suspension of 1×10^7 PC3 cells in physiological saline (100 µl) into left flanks of mice. After 3 weeks, *in vivo* CL imaging was achieved. Acute inflammation was induced in Balb/C mice (5 weeks old, Orient Bio Inc., Korea) as previously described.[1] In brief, inflammation was elicited with intra-articular injection of LPS (Sigma-Aldrich Co.). *In vivo* CL imaging was carried out 48 h after LPS treatment. For chronic inflammation model, the collagen-induced arthritis (CIA) mice were prepared. CIA was induced in DBA1/J mice as previous described.[2, 3] Male DBA1/J mice (5 weeks old) were obtained from SLC Inc. (Shizuoka, Japan). To induced CIA, 200 µg of bovine type II collagen (CII, 2 mg/ml, Chondrex, Redmond, WA, USA) emulsified in 100 µl of Complete Freund's Adjuvant (4 mg/ml, Chondrex) were injected intradermally into the base of the tail. The second immunization was boosted 21 days later with CII emulsified in Incomplete Freund's Adjuvant. *In*

vivo CL imaging was obtained 40 days after the primary immunization. The control groups were prepared by injecting the L012, PEG-QDs, and HNPs subcutaneously into the normal mice.

S2. Supporting Figures

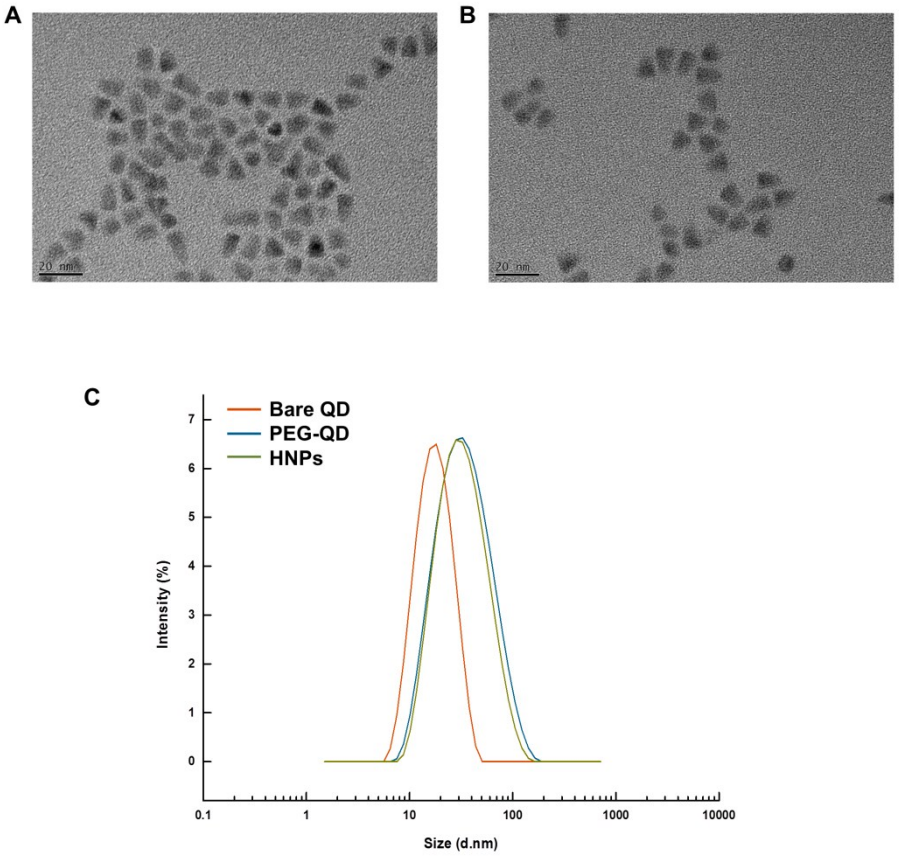

Fig. S1 TEM images of (A) PEG-QD and (B) HNPs and (c) size distribution of bare QDs before PEGylated (orange) PEG-QD (blue) and HNPs (green). Size distribution is measured using zetasizer ZS90. Both TEM images are obtained without the negative staining step. The size distribution shows the size difference albeit the TEM images can not revealed the difference.

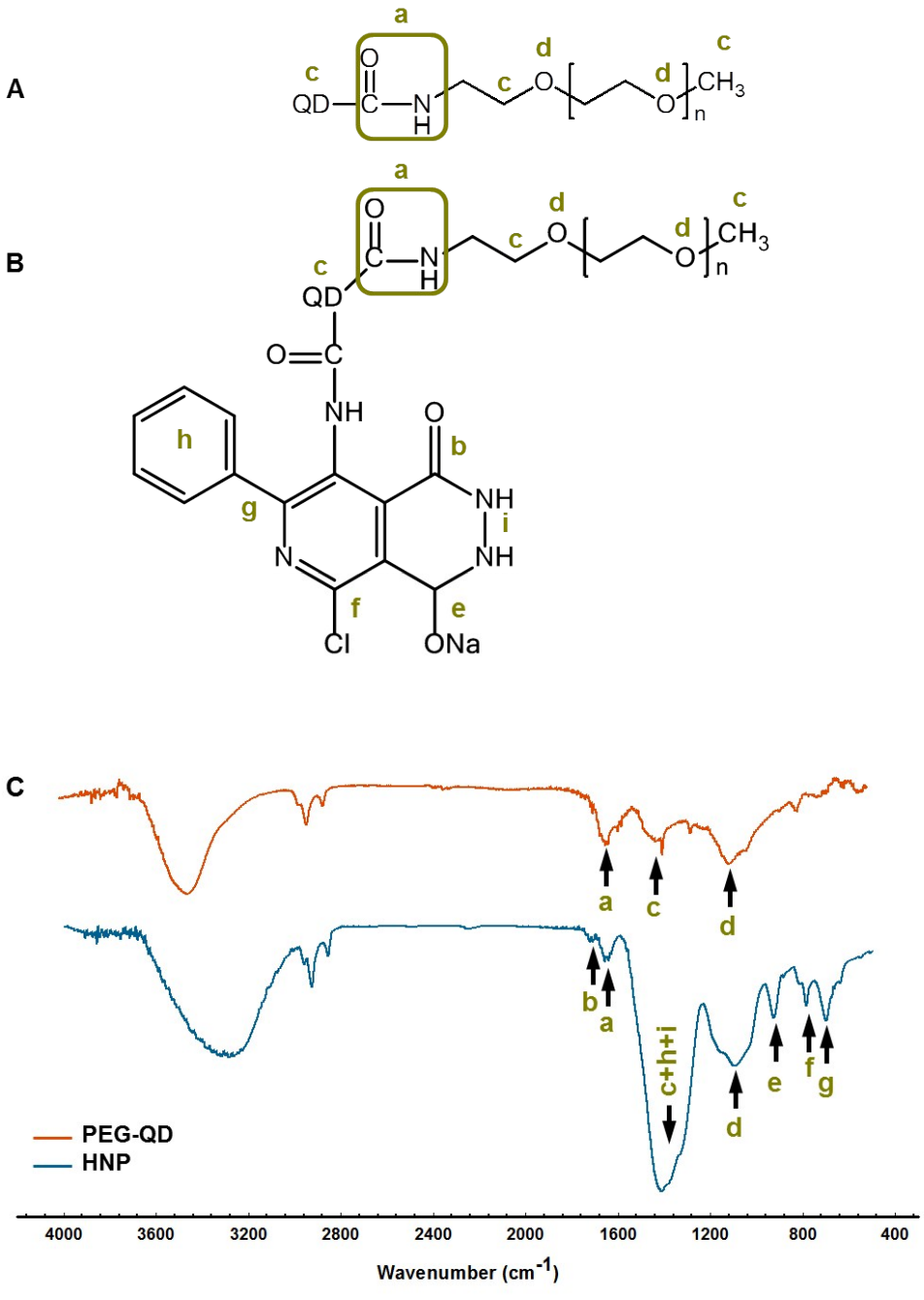
A
B
C
PEG-QD
HNP
Wavenumber (cm-1)
4000
3600
3200
2800
2400
2000
1600
1200
800
400

Fig. S2 Simplified chemical structures of (A) PEG-QD and (B) HNPs. (C) FT-IR spectra of PEG-QD (orange line) and HNPs (blue line). The alphabets which indicate specific bond in (A) and (B) are corresponding to those of (C).

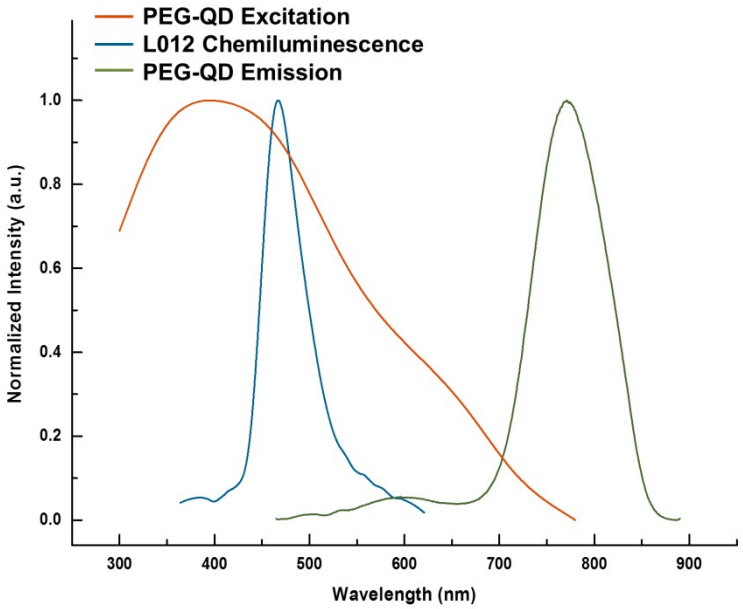

Fig. S3 Fluorescence spectra of PEG-QD and chemiluminescence spectra of L012. Orange line is excitation of PEG-QD and green line is emission of PEG-QD. Chemiluminescence spectrum (blue line) was measured 15 minutes after adding hydrogen peroxide.

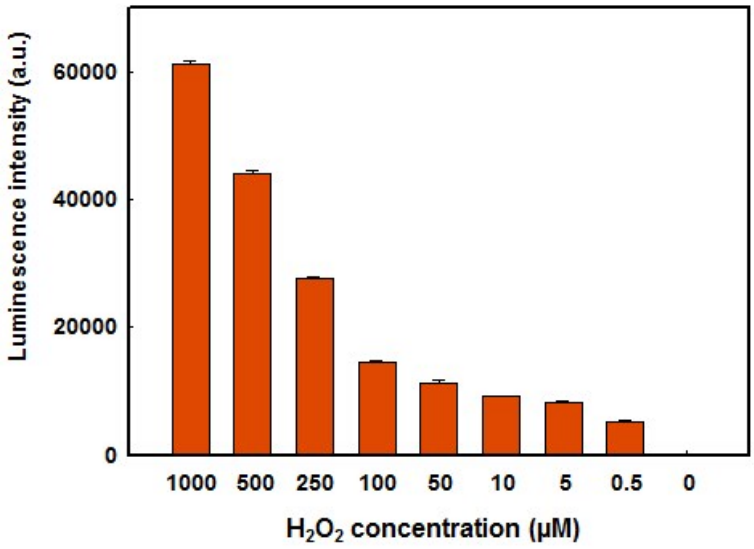

Fig. S4 Quantified luminescence intensity of HNPs according to various concentrations of hydrogen peroxide.

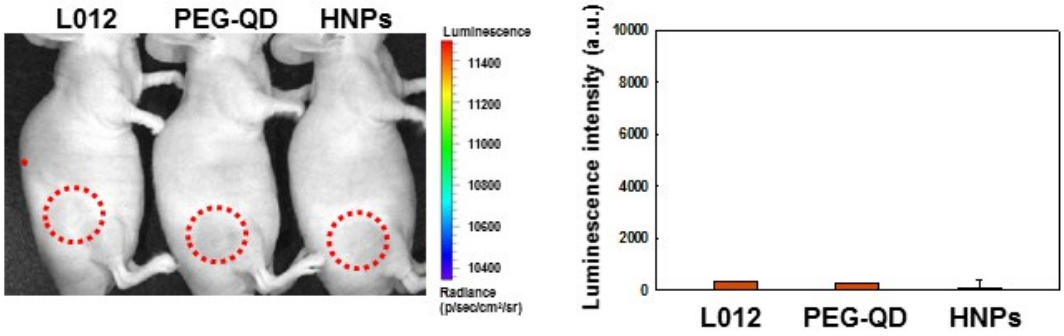

Fig. S5 Luminescent signals in normal mice. The error bars represent standard deviation (n=3).

References

(1) Lee, Y.-D.; Lim, C.-K.; Singh, A.; Koh, J.; Kim, J.; Kwon, I. C.; Kim, S., Dye/Peroxalate Aggregated Nanoparticles with Enhanced and Tunable Chemiluminescence for Biomedical Imaging of Hydrogen Peroxide. *ACS Nano*. **2012**, 6, 6759-6766.

(2) Heo, R.; Park, J.-S.; Jang, H. J.; Kim, S.-H.; Shin, J. M.; Suh, Y. D.; Jeong, J. H.; Jo, D.-G.; Park, J. H., Hyaluronan nanoparticles bearing γ-secretase inhibitor: In vivo therapeutic effects on rheumatoid arthritis. *J. Controlled Release.* **2014,** 192, 295-300.

(3) Shin, J. M.; Kim, S.-H.; Thambi, T.; You, D. G.; Jeon, J.; Lee, J. O.; Chung, B. Y.; Jo, D.-G.; Park, J. H., A hyaluronic acid-methotrexate conjugate for targeted therapy of rheumatoid arthritis. *Chem. Commun.* **2014,** 50, 7632-7635.