Electronic Supporting Information

Label-free nanoprobe for antibody detection through antibody catalysed water oxidation pathway

Kyeonghye Guk[a,c], Hyeran Kim[b], Yujeong Kim[a,c], Taejoon Kang[a,b,c], Eun-Kyung Lim[a,b,c]* and Juyeon Jung[a,c]*

[a]Hazards Monitoring BioNano Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea.

[b]BioNano Health Guard Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Republic of Korea.

[c]Major of Nanobiotechnology and Bioinformatics, University of Science and Technology, Daejeon 34113, Republic of Korea.

E-mail: jjung@kribb.re.kr; eklim1112@kribb.re.kr

Reagents and chemicals

4-(Dimethylamino)pyridine) (DMAP), poly(ethylene glycol) methyl ether (mPEG) (MW 5000), N,N'-dicyclohexylcarbodiimide (DCC), 1,4-dioxane, acetone, anti-human IgG, anti-human IgE and anti-human IgM were purchased from Sigma Aldrich (USA). Chlorin e6 (Ce6) and coumarin boronate were obtained from 4Chem Laboratory (Korea). Singlet oxygen sensor green reagent (cat. no. S36002) was purchased from Thermo Fisher Scientific. A hydrogen peroxide colorimetric detection kit (prod. no. ADI-907-015) was obtained from Enzo Life Science. All chemicals and reagents were of analytical grade and were used without further purification.

Synthesis of mPEG-Chlorin e6

DMAP, mPEG (0.1 mmol), chlorin e6 (0.15 mmol), and N,N'-dicyclohexylcarbodiimide (0.2 mmol) were dissolved in 40 mL of 1,4-dioxane to conjugate the hydroxyl group of mPEG to the carboxyl group of chlorin e6.[1, 2] This mixture was reacted under magnetic stirring at room temperature for 24 h. After the reaction, the product was purified by dialysis against excess deionized water (DW) for 7 days, with a membrane (molecular weight cut off: 3500 Da) to eliminate excess unreacted reagents. The resultant products (mPEG-Chlorin e6) were dried under a vacuum and stored for later use. Their chemical structure was confirmed by FT-IR and ^{1}H-NMR spectra with $CDCl_3$ as a solvent.

Nanoprobe preparation

The coumarin boronate-loaded nanoprobes were prepared through a precipitation method. 2 mg of coumarin boronate was dissolved in 2 mL of acetone and rapidly injected into 40 mL of

DW containing 25 mg of mPEG-Chlorin e6 under magnetic stirring. This reactant was stirred to evaporate the acetone for 24 h at room temperature. Subsequently, the solution was centrifuged at 15,000 rpm for 45 min three times. After the supernatants were removed, the precipitated nanoprobes were re-dispersed in 2 mL of DW.

Nanoprobe characterization

The size and morphology of the nanoprobes were determined by dynamic laser scattering (DLS) (ELS-Z, Otsuka Electronics) and scanning electron microscopy (SEM), respectively. The chlorin e6 of the nanoprobes was measured using UV/Vis spectroscopy.

Measurement of the singlet oxygen generation by the nanoprobe

The singlet oxygen generation of the nanoprobe was measured using the commercial singlet oxygen sensor reagent for detecting singlet oxygen. Various nanoprobe concentrations were prepared in a 96-well plate and then irradiated with UV light (λ_{ex}: 365 nm) for 10 min. After detection reagent treatment each reaction was further incubated for different times (10, 20 and 30 min), and the fluorescence was measured at 504 nm (excitation) and 525 nm (emission) using a microplate reader (Infinite 200 PRO NanoQuant, TECAN).

Measurements of the immunoglobulin G (IgG) antibody using the nanoprobe

First, we measured the hydrogen peroxide (H_2O_2) concentrations produced by the nanoprobe, by using a commercial kit (hydrogen peroxide colorimetric detection kit). The IgG antibodies were incubated with the nanoprobe at its different concentrations and then irradiated with UV light (ex: 350 nm) for 10 min. Subsequently, the reagent for H_2O_2 detection was added to each well and further incubated for 0.5 h, 1 h, 1.5 h or 2 h. Next, the optical densities (O.D.s) were measured at 550 nm using a microplate reader, as recommended by the manufacturer. Then, we detected the various antibodies (IgG, IgM and IgE) by using only nanoprobes. Each antibody was added to each well of the plates at various concentrations and incubated overnight at 4 °C. After incubation, nanoprobe (1, 2 and 20 mM) was added to each well and then exposed to UV light (λ_{ex}: 365 nm) for 10 min. The generated fluorescence of the nanoprobe was measured with a microplate reader (λ_{ex}: 332 nm and λ_{em}: 454 nm).

References

1. E. K. Lim, J. Yang, C. P. Dinney, J. S. Suh, Y. M. Huh and S. Haam, Biomaterials, 2010, 31, 9310-9319.

2. E.-K. Lim, E. Jang, J. Kim, T. Lee, E. Kim, H. S. Park, J.-S. Suh, Y.-M. Huh and S. Haam, J. Mater. Chem. 2012, 22, 17518-17524.

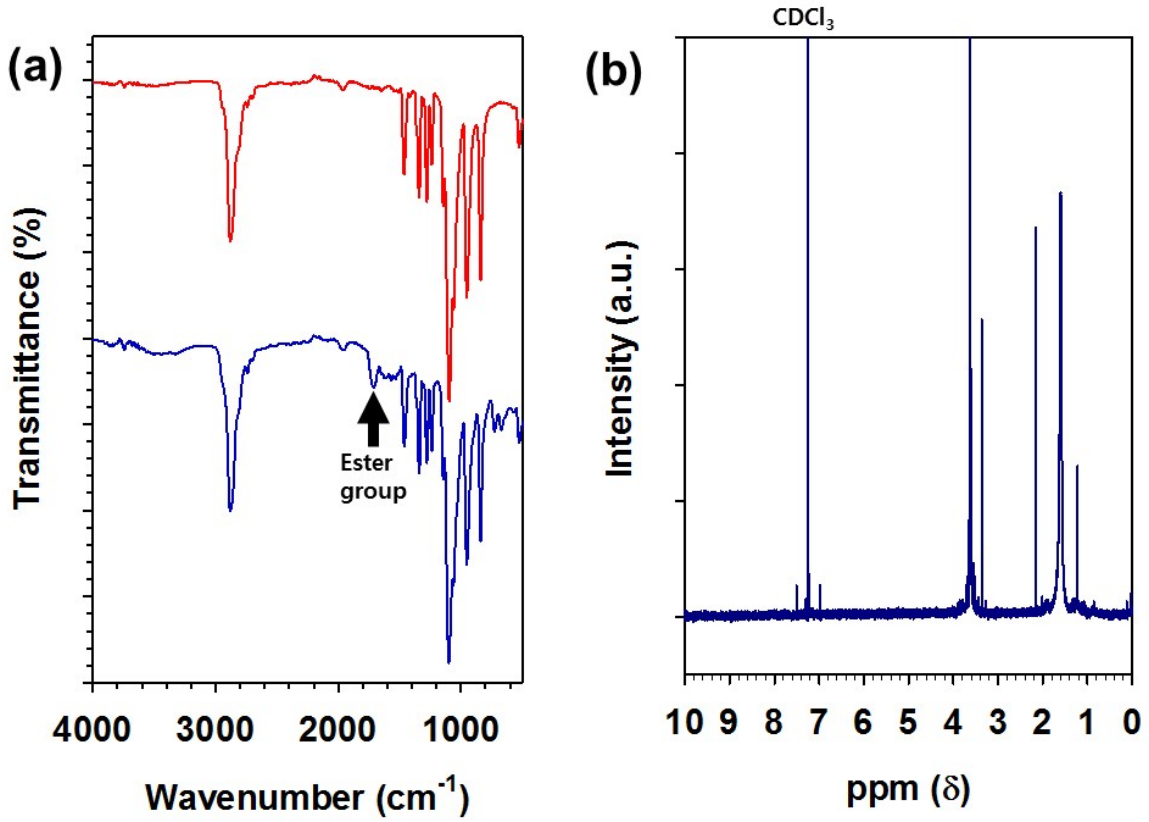

Figure S1. Characteristics of mPEG-Chlorin e6. (a) FT-IR spectra (mPEG-Chlorin e6: blue and mPEG: red) and (b) ^{1}H-NMR spectrum in $CDCl_3$.

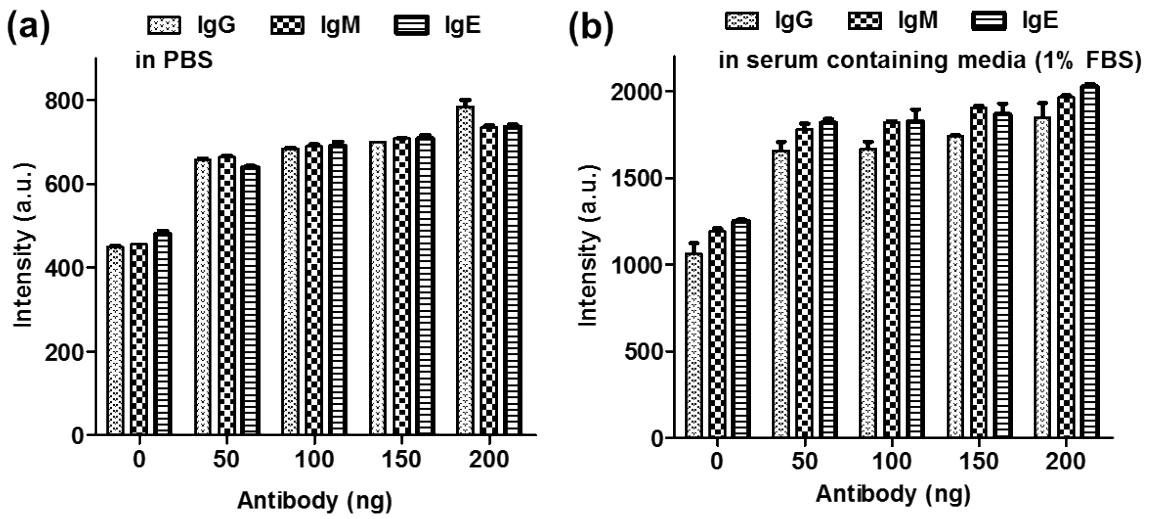

Figure S2. The effects of the nanoprobe on the detection of various antibodies. Fluorescence intensities of the nanoprobe with different amounts of various antibodies (a) in PBS and (b) in serum containing media (1% FBS) .