# **Electronic Supplementary Information (ESI)**

## **Oxidation-Triggered Aggregation of Gold Nanoparticles for Naked-Eye**

## **Detection of Hydrogen Peroxide**

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#### Materials and instrument

Ammonium chloride, anhydrous dichloromethane (DCM), anhydrous dimethylformamide (DMF), 2,6bis-(hydroxymethyl)-p-cresol, 4-bromomethylphenylboronic acid pinacol ester, *tert*-butyldimethylsilyl chloride, 1,1'-carbonyldiimidazole, copper(II) sulfate pentahydrate, N,N'-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), gold(III) chloride trihydrate, hydrogen peroxide solution, imidazole, lipoic acid, mPEG<sub>5000</sub>-azide, poly(ethylene glycol) methyl ether (M.W. 5000), potassium carbonate, propargylamine, sodium ascorbate, sodium citrate, *p*-toluenesulfonic acid monohydrate were purchased from Sigma Aldrich and used without further purifications.

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were measured on a JEOL ECASL400 spectrometer. Mass spectrometry was measured on a ThermoFinnigan LCQ Fleet MS spectrometer. UV-vis spectra were recorded using a Shimadzu UV-3600 UV–vis–NIR spectrophotometer.

#### **Organic synthesis**

**Compound 1:** 2,6-Bis-(hydroxymethyl)-p-cresol (1.68 g, 10.0 mmol) and imidazole (1.54 g, 22.7 mmol) were dissolved in anhydrous DMF (7 mL). The solution was stirred at 0 °C, to which *tert*-butyldimethylsilyl chloride (3.31 g, 22.0 mmol) dissolved in anhydrous DMF (5 mL) was added. The solution was stirred at room temperature for 2 h, and then it was diluted with ether and washed with water for 3 times. Thereafter, it was dried with sodium sulfate, and ether was removed. The residue was purified through chromatography (hexane : ethyl acetate = 95 : 5) to afford colorless oil (3.65g, Yield 92.6%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): 8.07 (s, 1H), 6.97 (s, 2H), 4.89 (s, 4H), 2.32 (s, 3H), 1.01 (s, 18H), 0.19 (s, 12H). <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>): 150.97, 128.28, 126.24, 125.80, 63.06, 25.93, 20.72, 18.35, -5.37. ESI-MS: m/z calcd for C<sub>21</sub>H<sub>40</sub>O<sub>3</sub>Si<sub>2</sub>: 396.25, found: 395.05 [M-H]<sup>+</sup>.



Compound 5



**Compound 2:** Compound **1** (0.83 g, 2.0 mmol) was dissolved in anhydrous DMF (4 mL), to which potassium carbonate (0.35 g, 2.5 mmol) was added, and the solution was stirred at 0 °C for 10 min. 4-Bromomethylphenylboronic acid pinacol ester (0.65 g, 2.0 mmol) was added to the solution and the reaction solution was stirred at room temperature overnight. Thereafter, the solution was diluted with ether and washed with saturated ammonium chloride aqueous solution and brine solution. The organic phase was collected and dried over magnesium sulfate. The raw product was purified through chromatography (hexane : ethyl acetate = 95 : 5) to afford slightly yellow oil (1.15 g, Yield 80%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): 7.85 (d, 2H), 7.43 (d, 2H), 7.20 (s, 2H), 4.91 (s, 2H), 4.72 (s, 4H), 2.36 (s, 3H), 1.38 (s, 12H), 0.94 (s, 18H), 0.09 (s, 12H). <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>): 151.10, 140.78, 135.03, 133.80, 133.66, 127.90, 126.95, 83.82, 76.15, 60.36, 26.02, 24.89, 21.21, 18.44, -5.23. ESI-MS: m/z calcd for C<sub>34</sub>H<sub>57</sub>BO<sub>5</sub>Si<sub>2</sub>: 612.38, found: 611.27 [M-H]<sup>+</sup>.

**Compound 3:** Compound **2** (1.15 g, 1.9 mmol) was dissolved in MeOH (6 mL), and then *p*-toluenesulfonic acid monohydrate (0.07 g, 0.4 mmol) was added. The reaction solution was stirred at room temperature overnight, after which MeOH was removed. The residue was subjected to chromatography (hexane : ethyl acetate = 1 : 1) to afford colorless oil (0.62 g, Yield 85.6%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): 7.73 (d, 2H), 7.30 (d, 2H), 7.05 (s, 2H), 4.72 (s, 2H), 4.51 (s, 4H), 2.17 (s, 3H), 1.29 (s, 12H). <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>): 151.96, 140.26, 135.02, 133.98, 133.84, 129.01, 127.06, 83.86, 76.44, 59.95, 24.79, 24.62. ESI-MS: m/z calcd for C<sub>22</sub>H<sub>29</sub>BO<sub>5</sub>: 384.27, found: 790.96 [2M+ Na]<sup>+</sup>.

**Compound 4:** Compound **3** (0.61 g, 1.6 mmol), lipoic acid (0.16 g, 0.8 mmol), DMAP (0.03 g, 0.2 mmol) were dissolved in DCM (7 mL). The solution was stirred at 0 °C for 10min, and then DCC (0.18 g, 0.9 mmol) dissolved in DCM (1.6 mL) was added dropwise. The reaction solution was then stirred at room temperature overnight. After removing the generated solids, DCM was removed and the residue was purified through chromatography (hexane : ethyl acetate = 5 : 1) to afford yellow oil (0.23g, Yield 50%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): 7.80 (d, 2H), 7.40 (d, 2H), 7.19 (s, 1H), 7.11 (s, 1H),

5.13 (s, 2H), 4.91 (s, 2H), 4.62 (s, 2H), 3.44-3.49 (m, 1H), 3.02-3.15 (m, 2H), 2.28-2.42 (m, 7H), 1.79-1.87 (m, 2H), 1.33-1.43 (m, 16H). <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>): 173.30, 153.16, 140.05, 135.10, 134.30, 130.66, 130.37, 129.03, 126.96, 83.89, 76.90, 61.60, 60.51, 56.26, 40.17, 38.47, 34.54, 34.09, 28.68, 24.90, 24.81, 24.64. ESI-MS: m/z calcd for  $C_{30}H_{41}BO_6S_2$ : 572.58, found: 573.08 [M+H]<sup>+</sup>, 590.12 [M+NH<sub>4</sub>]<sup>+</sup>.

**Compound 5:** Compound **4** (0.21 g, 0.4 mmol) was dissolved in anhydrous DCM (2 mL), and then 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol) was added. The reaction solution was stirred at room temperature overnight. Thereafter, the solution was diluted with DCM and washed with brine for 3 times and dried over magnesium sulfate. After removing DCM, the residue was purified through chromatography (hexane : ethyl acetate = 2 : 1) to afford yellow oil (0.12 g, Yield 50%). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): 8.09 (s, 1H), 7.82 (d, 2H), 7.41 (d, 2H), 7.37 (s, 1H), 7.25 (s, 2H), 7.04 (s, 1H), 5.40 (s, 2H), 5.19 (s, 2H), 4.99 (s, 2H), 3.50-3.53 (m, 1H), 3.06-3.15 (m, 2H), 2.34-2.44 (m, 6H), 1.84-1.89 (m, 1H), 1.62-1.66 (m, 4H), 1.36-1.47 (m, 14H). <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>): 173.22, 154.23, 148.57, 139.60, 137.14, 135.21, 134.63, 132.72, 131.88, 130.67, 129.84, 127.71, 126.76, 117.21, 83.94, 74.77, 65.20, 61.35, 60.44, 56.31, 40.24, 38.52, 34.61, 34.12, 28.76, 24.95, 21.10. ESI-MS: m/z calcd for C<sub>34</sub>H<sub>43</sub>BN<sub>2</sub>O<sub>7</sub>S<sub>2</sub>: 666.26, found: 666.93 [M+H]<sup>+</sup>, 689.21 [M+Na]<sup>+</sup>.

**Compound 6:** To synthesize compound **6**, compound **5** was used without purification by chromatography. Compound **4** (0.51 g, 0.9 mmol) was dissolved in anhydrous DCM (4 mL), and 1,1'-carbonyldiimidazole (0.29 g, 1.8 mmol) was added. The solution was stirred at room temperature overnight. After diluted with DCM, the solution was washed with brine for 3 times. The DCM solution was then dried over magnesium sulfate and DCM was removed under reduced pressure. Thereafter, anhydrous DCM (4 mL) was added to dissolve the residue (compound **5**), to which propargylamine (1.11 mL, 17.8 mmol) was added, and the solution was stirred at room temperature overnight. The solution was washed with water and brine, and then dried over magnesium sulfate. The raw product was purified through chromatography to afford yellow oil (0.32 g, 0.49 mmol, Yield 55%). <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>): 7.74 (d, 2H), 7.49 (d, 2H), 7.24 (d, 2H), 5.10 (d, 4H), 4.95 (s, 2H), 3.81 (s, 2H), 3.56-3.59 (m, 1H), 3.12-3.20 (m, 3H), 2.33-2.42 (m, 6H), 1.83-1.88 (m, 1H), 1.51-1.64 (m, 4H), 1.28-1.40 (m, 14H). <sup>13</sup>C NMR (400MHz, CDCl<sub>3</sub>): 173.27, 155.83, 153.91, 140.01, 135.18, 134.31, 131.60, 129.77, 129.45, 127.00, 83.94, 71.78, 62.35, 61.55, 56.33, 53.53, 40.24, 38.54, 34.66, 34.15, 28.77, 25.00, 24.96, 24.71, 22.44, 20.90. ESI-MS: m/z calcd for  $C_{34}H_{44}BNO_7S_2$ : 653.27, found: 654.20 [M+H]<sup>+</sup>.

Synthesis of lipoic-phenylboroante-mPEG<sub>5000</sub>: Compound 6 (0.20 g, 0.3 mmol) and mPEG<sub>5000</sub>-azide (1.55 g, 0.3 mmol) were dissolved in DMF (10 mL). Then, CuSO<sub>4</sub>·5H<sub>2</sub>O (0.16 g, 0.6 mmol) dissolved in water (1 mL) was added. Sodium ascorbate (0.37 g, 1.9 mmol) dissolved in water (1 mL) was slowly added under vigorous stirring. The flask of the solution was then sealed and the solution was stirred for further 3 days. Thereafter, DMF and water were removed under reduced pressure at 40 °C. DCM (30 mL) was then added to dissolve the residue, and insoluble solids were removed by filtration. The raw product was then purified via chromatography (hexane/ethyl acetate 50% v/v and then MeOH/DCM 10% v/v) to afford white solid wax (1.24 g, 0.06 mmol, Yield 17%). <sup>1</sup>H NMR (400MHz,

CDCl<sub>3</sub>): 7.85 (d, 2H), 7.40 (d, 2H), 7.17 (s, 3H), 5.17 (m, 4H), 4.90 (s, 2H), 3.79-3.81 (s, 6H), 3.62 (s, 1916H), 3.35 (s, 12H), 3.00-3.18 (m, 3H), 2.28-2.32 (m, 6H), 1.80-1.90 (m, 1H), 1.56-1.70 (m, 4H), 1.17-1.51 (m, 14H). According to the integral of NMR proton peaks, the content of lipoic-phenylboroante-mPEG<sub>5000</sub> is about 25%, and the rest of 75% is mPEG-azide. Since mPEG azide does not bind to AuNPs, it does not interfere with the absorption of lipoic-phenylboroante-mPEG<sub>5000</sub> to AuNPs. When preparing lipoic-phenylboroante-mPEG<sub>5000</sub> stock solution, the quantity of lipoic-phenylboroante-mPEG<sub>5000</sub> was adjusted according to the content of the polymer. AuNP solution could resist aggregation in saturated brine after the addition of the stock solution, proving that lipoic-phenylboroante-mPEG<sub>5000</sub> was adsorbed onto AuNPs.

Synthesis of  $H_2O_2$ -responsive AuNPs: Citrate stabilized AuNPs were synthesized by the following procedure: HAuCl<sub>4</sub>·3H<sub>2</sub>O (0.07 g, 0.17 mmol) was firstly dissolved in deionized water (386 mL), after which the solution was heated to boiling. Under vigorous stirring, sodium citrate di-hydrate (0.23 g, 0.8 mmol) dissolved in deionized water (4 mL) was injected to the above solution. Thereafter, the reaction was maintained boiling for 15mins before it was cooled down to room temperature. The size of as-synthesized AuNPs was about 14nm.

 $H_2O_2$ -responsive AuNPs were prepared by the following procedure: the as-synthesized citrate stabilized AuNPs (2 mL) was centrifuged, and the supernatant was removed. AuNPs were then dissolved in DMF (2 mL). Lipoic-phenylboroante-mPEG<sub>5000</sub> in DMF (10 µL, 0.019 M) was added and the solution was stirred for a few hours. Subsequently, 1-hexanethiol in DMF (10 µL, 9% v/v) was added. The obtained AuNP solution was stirred at room temperature for a few hours and subjected to centrifugation to afford functionalized AuNPs. These AuNPs were washed by centrifugation-washing cycles for a few times to remove excess amount of ligands and finally dissolved in PBS 7.4 solution for incubation with  $H_2O_2$ .



**Fig. S1** (a-d) Evolution for <sup>1</sup>H NMR spectra of compound **6** in the reaction with  $H_2O_2$ . Compound **6** (9.76 mM) in DMSO-d<sub>6</sub> (456 µL) and deuterated PBS 7.4 solution (124 µL),  $H_2O_2$  ( $H_2O$  solution, 10M, 6 µL,  $H_2O_2$  concentration of 96.8mM), compound **6** :  $H_2O_2 = 1 : 10$ . Inset: enlargement of the proton peak at 4.52 ppm.



**Fig. S2** Gradual aggregation and color change of functionalized AuNP solutions incubated with different concentrations of  $H_2O_2$  at 37 °C. B: AuNP solution without  $H_2O_2$ . 1-14: 10  $\mu$ M, 20  $\mu$ M, 40  $\mu$ M, 70  $\mu$ M, 100  $\mu$ M, 120  $\mu$ M, 160  $\mu$ M, 200  $\mu$ M, 300  $\mu$ M, 400  $\mu$ M, 600  $\mu$ M, 800  $\mu$ M, 1 mM, 2 mM of  $H_2O_2$ . Time: a, 0h; b, 3h 45min; c, 6h 31min; d, 9h 11min; e, 16h 24min; f, 20h 16min; g, 24h; h, 28h; i, 43h 33min; j, 69h.





**Fig. S3** UV-vis absorption spectra of functionalized AuNPs treated with a serials of  $H_2O_2$  concentrations. Duration of incubation: (a) 3h 45min; (b) 6h 31min; (c) 16h 24min. Corresponding charts below the UV-vis absorption spectra show the detail data of the absorption intensities at 527 nm and 650 nm and the ratio between them.



**Fig. S4** Aggregation of AuNPs at 34 h of incubation with a serial of  $H_2O_2$  solutions at 25 °C. (a): photograph of the color change of AuNPs upon  $H_2O_2$  additions. (b): UV-vis absorption spectra of AuNPs upon  $H_2O_2$  additions. (c,d): data of the absorption intensity ratios (from blank sample to 300  $\mu$ M sample) and linear fitting of the data (from 40  $\mu$ M sample to 200  $\mu$ M sample). (e): chart of the related data.



**Fig. S5** (a) UV-vis absorption spectra for the aggregation experiment of AuNPs modified with two different amounts of lipoic-phenylboronate-mPEG<sub>5000</sub> ligand upon the additions of 100  $\mu$ M and 600  $\mu$ M H<sub>2</sub>O<sub>2</sub> at 37 °C. (b) Corresponding color change of the AuNP solutions. Incubation time was 3 h. 1: 0.0185 M PEG ligand in 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>, blue line; 2: 0.0185 M PEG ligand in 600  $\mu$ M H<sub>2</sub>O<sub>2</sub>, pink line; 3: 0.074 M PEG ligand in 100  $\mu$ M H<sub>2</sub>O<sub>2</sub>, black line; 4: 0.074 M PEG ligand in 600  $\mu$ M H<sub>2</sub>O<sub>2</sub>, red line.