Supporting Information

*Ginkgo Biloba* leaf polysaccharide stabilized palladium nanoparticles with enhanced peroxidase-like property for the colorimetric detection of glucose

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Materials and methods

1.1 Materials

H$_2$O$_2$ solution (30 wt.%), terephthalic acid, Na$_2$PdCl$_4$, and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Aladdin. Glucose, maltose, fructose, lactose, sodium carbonate, sodium bicarbonate, sodium acetate, acetic acid and hydrochloric acid were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd. Glucose oxidase (GOD) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Dialysis bags (MWCO=8000-14000 Da) were purchased from Spectrum Laboratories INC.

1.2 Extraction of GBLP

The GBLP was extracted according to previous report with slight modification. Briefly, dried *Ginkgo Biloba* leaf powder was extracted with water at 85 °C (1:25 (w/v), 3 h, twice). The extracted solution was obtained by centrifugation, and H$_2$O$_2$ was added to remove the pigment at 50 °C (1:10 (v/v), 2 h). Then, Sevage reagents including chloroform and butyl alcohol (5:1 (v/v)) were added to remove proteins. The obtained solution was dialyzed against water and freeze-dried to obtain GBLP.

1.3 Synthesis of *Ginkgo Biloba* leaf polysaccharide stabilized palladium nanoparticles (Pd$_n$-GBLP NPs)

6.45 mL of 2 mM Na$_2$PdCl$_4$ aqueous solution was added to 11 mL of 1 mg/mL GBLP aqueous solution at 60 °C and stirred for 12 h at 500 r/min. The molar ratio of Pd to GBLP was 91:1. The solution was dialyzed against water to obtain Pd$_{91}$-GBLP NPs. The others Pd$_n$-GBLP NPs (n=41, 68, 137) were prepared with the same procedure.

1.4 Characterization
UV-Vis spectra were measured using UV-TU1810PC spectrophotometer. FTIR was measured using E55-FRA106 of Bruker. The sizes of the Pd NPs inside of Pdn-GBLP NPs were characterized by HT7700 TEM. The hydrodynamic size and zeta potential of Pdn-GBLP NPs were characterized by DLS technology. Fluorescence spectroscopy was measured using F-7000 of HITACHI. The concentration of Pd was measured by inductively coupled plasma-mass spectrometry (ICP-MS).

1.5 Biocompatibility
HeLa cells were seeded in 96-well tissue culture plates (10^4 cells/well) and cultured in 200 μL high-glucose DMEM medium containing 10% FBS at 37°C. After one day, the medium was replaced by GBLP and Pd91-GBLP NPs dissolved in high-glucose DMEM medium with different concentrations. After 24 h, the medium was replaced by 100 μL of high-glucose DMEM medium with 0.5 mg/mL MTT. After 4 h, the medium was replaced by 150 μL DMSO and measured using SpectraMaxM2. Cell viability was calculated by comparison absorbance of samples with control ones. Inverted fluorescence microscope (TS100) was used to obtain the cell morphology.

1.6 Evaluation of peroxidase-like activity
The catalytic oxidation of TMB using Pd91-GBLP NPs was carried out at 25 °C in the presence of H2O2. All TMB was dissolved in HAc-NaAc buffer solution (0.2 M). 100 μL of Pd91-GBLP NPs (5 μM), 350 μL HAc-NaAc buffer solution (0.2 M, pH 4.0), and 800 μL of TMB (0.6 mM, pH 4.0) were incubated for 5 min. 150 μL of H2O2 (0.3 M) was added for 10 min. The control groups were also measured using UV-Vis spectra15. To explore the effect of temperature and pH on the catalytic activity of Pd91-GBLP NPs, The mixture of NaAc buffer (0.2 M, pH 4.0), H2O2 (0.3 M), TMB (0.6 mM), and Pd91-GBLP NPs (5 μM) were reacted at different temperatures from 10 °C to 70 °C and a different pH range from 1.0 to 12.0, respectively.

1.7 Michaelis-Menten kinetic analysis
The kinetic measurements were performed by using 50 μL of Pd91-GBLP NPs (5 μM) with TMB (0.6 mM) as substrate, or H2O2 (0.3 M). Under the optimum condition, the concentration of one substrate was fixed when the other was changed, and the time scan of catalytic process was determined by UV-Vis spectra. The obtained curve was fitted through Michaelis-Menten kinetic analysis.

1.8 Glucose detection
To obtain a standard curve for glucose detection, 100 μL of glucose oxidase (5 mg/mL) and 200 μL glucose solution were incubated at 37 °C for 30 min. Then 900 μL TMB (0.6 mM) and 200μL Pd91-GBLP NPs (5 μM) were mixed and incubated at 40 °C for 10 min. The two reaction solutions were mixed together and reacted for 30 minutes at 40 °C, and the resulting mixture was measured by UV-Vis spectra.
In order to assess the selectivity for detecting glucose in the assay, glucose (5 mM), maltose (10 mM), lactose (10 mM) and fructose (10 mM) were used as the control group for the detection, and
the above method was used for the detection. At the same time, the method was used to measure the glucose concentration of blood as a real sample.

2. Results and Discussion

The GBLP and Pd91-GBLP NPs were further characterized by FTIR. As shown in Fig.S1, 3414 cm\(^{-1}\) was attributed to the O-H stretching vibration, 2921 cm\(^{-1}\) was due to C-H stretching vibrations, 1733 cm\(^{-1}\) was assigned to C=O stretching vibration, 1634 cm\(^{-1}\) corresponded to -OH flexural vibration, 1421 cm\(^{-1}\) was due to =CH\(_2\) deformation absorption, 1021 cm\(^{-1}\) was attributed to C-H stretching vibration. The spectrum of Pd91-GBLP NPs was similar with GBLP.

![FTIR spectra of (a) GBLP and (b) Pd91-GBLP NPs](image)

**Fig.S1.** FTIR spectra of (a) GBLP and (b) Pd91-GBLP NPs

References

1. Yan, Z.; Fan, R.; Yin, S.; Zhao, X.; Liu, J.; Li, L.; Zhang, W.; Ge, L. Protective effects of Ginkgo biloba leaf polysaccharide on nonalcoholic fatty liver disease and its mechanisms. *Int. J. Biol. Macromol.* 2015, 80, 573-580.