

Supplementary Information

Highly sensitive free radical detection by nitron-functionalized gold nanoparticles

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Experimental section

Materials.

All starting materials were purchased from Sigma-Aldrich. All solvents were distilled and dried according to standard procedures. TLC analyses were performed on sheets precoated with silica gel 60F254 (Merck). Flash chromatographic experiments were carried out on Merck silica gel Geduran Si 60 (0.063-0.200 mm), and size exclusion chromatographic experiments were carried out on Sephadex LH-20 (Amersham Biosciences). Mass spectra were recorded on a Shimadzu LCMS-2010. UV spectra were recorded on a Hitachi U-3310 apparatus. The ¹H spectra were recorded on a Bruker ESP-300 spectrometer. The concentrations of gold in the AuNPs were measured using an inductively coupled plasma mass spectrometer (ICP-MS) (Anilent 7500a; Agilent Technologies, Santa Clara, CA, USA). Chemical shifts were given in ppm relative to tetramethylsilane using the deuterium signal of the solvent (CDCl₃) as a heteronuclear reference for ¹H and ¹³C. Abbreviations used for signal patterns are as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet.

Thermal gravimetric analysis (TGA) (~5 mg sample tested) was conducted in an N₂ atmosphere (TGA) (flow rate 50 mL/min) with a TG/DAT Q5000 IR analyzer (TA Instruments, Inc.). All measurements were performed from room temperature with a heating rate of 10 °C/min to 900 °C. For TEM measurements, solution with a concentration of 1-2 mg/mL were prepared by dissolving the nanoparticles in methanol. A droplet of these nanoparticles was casted onto carbon-coated copper grids. The solvent was then allowed to evaporate slowly. TEM images were obtained at a magnification of 100 000 for the nanoparticles. All samples were recorded with a JEOL 100CX electron microscope operating at an acceleration voltage of 100 kV. The images were then analyzed by using Image J software (version 1.44). The Au-nanoparticle concentrations were assessed by quantitative inductively coupled plasma mass spectrometry (ICP-MS) measurement. Briefly, the Au-nanoparticles were digested in aqua fortis (nitric acid: hydrochloric acid 3:1). After adjusting the solution volume to 2 mL using 2% nitric acid and 1% hydrochloric acid (1:1), gold assays were performed using an ELAN DRC e ICP-MS instrument (Perkin Elmer, Massachusetts, USA).

The calculation method for evaluating the formula of Au@EMPO was as follows: The ratio among AuNPs and EMPO can be estimated by XPS (Table S1), ICP-MS (Table S2), TGA (Fig. S2). Then, assuming that the gold nanoparticle core is an ideal sphere, the volume of the AuNPs can be calculated according to the size (4.5 nm) of the nanoparticle core. Meanwhile, the volume of gold atom can be estimated according to the size (0.16 nm) of gold atom. Then according to the molecular ratios between gold atom and EMPO, the formula and the concentration of Au@EMPO can be obtained. Similarly, the formula and the concentration of Au@PEG3EMPO can also be obtained.

Preparation of CMPO

CMPO was synthesized and purified according to procedures described previously.¹

Synthesis of Au@PEG3EMPO

Au@PEG3 was synthesized according to our previous study.³ Then, EDC (34 mg 0.18 mM) and DMAP (21.9 mg 0.18 mM) were added to a solution of Au@PEG3 (20 mg) and CMPO (43 mg, 0.03 mM) in anhydrous DMF (10 mL) with stirring for 1 h at

0°C and then overnight at room temperature. The solution was evaporated and subsequent purification by column chromatography using sephadex LH-20 (methonal eluent) to remove the byproducts and excess CMPO. The pure nanoparticles (Au@PEG3EMPO) were stored as dry powder at -20°C until use. The represent formula of Au@PEG3EMPO is $\text{Au}_{1441} @ (\text{PEG3})_{572} \text{EMPO}_{331}$.

Hemolysis assay

Mice blood stabilized with EDTA was obtained from the Laboratory Animal Resources office of Peking Univeristy. The serum was removed from the blood by centrifugation and suction, and the red blood cells were then washed five times with sterile isotonic PBS solution. Following the last wash, the cells were diluted to 1/10 of their volume with sterile isotonic PBS solution.

The diluted RBC suspension (30 cm^{-3}) was then mixed with: a) 120 mm^3 of PBS as a negative control; b) 120 mm^3 nanopure water as a positive control; c) 120 mm^3 of a suspension of the Au@EMPO at concentrations ranging from 25 to $100 \mu\text{g cm}^{-3}$. The mixtures were then vortexed and then let to rest for 2 h at room temperature. After that time, the samples were centrifuged and the absorbances of the supernatants at 541 nm were measured in a Hitachi 3310 UV-Visible station. The percent hemolysis of each sample was calculated by dividing the difference in absorption between the sample and the negative control by the difference in absorption between the positive and the negative controls, and multiplying the resulting ratio by 100.

Spin Trapping Studies

ESR Measurements

ESR measurements were carried out on an ESR spectrometer equipped with high sensitivity resonator at room temperature. Unless otherwise indicated, the following acquisition parameters were used: microwave power, 12.9 mW; modulation amplitude, 0.1 mT; receiver gains, 1×10^3 - 2×10^3 ; scan time, 84 s; sweep width, 100 G. All spin trapping studies were carried out in a phosphate buffer (PBS) (0.1 M) at pH 7.0 containing 2 mM diethylene triamine pentaacetic acid (DTPA). Sample cells used were 200 μL quartz capillary tubes. Computer simulation of EPR spectra was carried out using the EPR simulation program (ROK\NEPR) developed by Prof. Rockenbauer.⁴

Spin Trapping of Hydroxyl Radical

Hydroxyl radical was generated by a Fenton system including FeSO_4 (2 mM), H_2O_2 (2 mM) and EDTA (2 mM) in phosphate buffer (0.1 M, pH 7.0). ESR spectra were recorded 40 s after mixing.

Superoxide Trapping

KO_2 /18-crown-6/DMSO system was used as a superoxide source. The nano spin traps Au@EMPO (27 μM) were mixed with a solution of 18-crown-6 (160 mM) and KO_2 (10 mM) in DMSO. EPR spectra were recorded 40s after mixing.

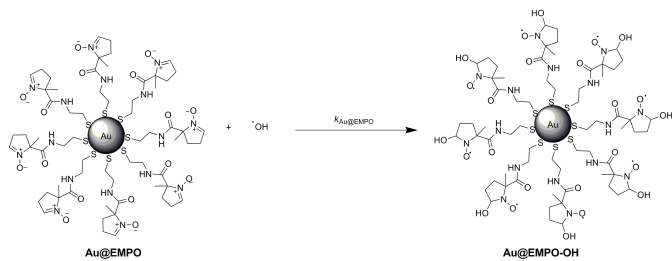
Kinetic Studies

Kinetic Studies of $\cdot\text{CH}(\text{CH}_3)\text{OH}$ Trapping

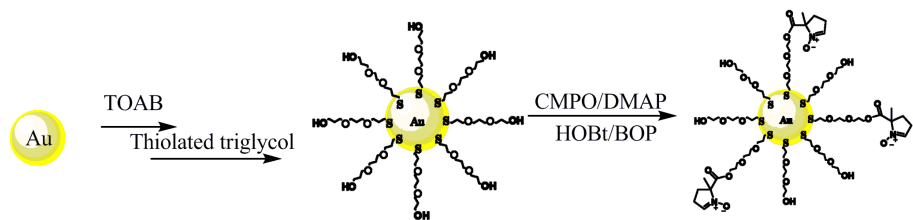
The C radical ($\cdot\text{CH}(\text{CH}_3)\text{OH}$) was generated by Fenton reaction including FeSO_4 (2 mM), H_2O_2 (2 mM), EtOH (v/v = 10%) and EDTA (1mM) in phosphate buffer (0.1 M, pH 7.0). All solutions were bubbled with nitrogen gas prior to irradiation. The second-order rate constants of Au@EMPO and EMPO with $\cdot\text{CH}(\text{CH}_3)\text{OH}$ were determined using PBN as a competitor. While a constant concentration of PBN (10 mM) was used, the concentration of Au@EMPO or EMPO was varied from 0 to 33.8 μM for Au@EMPO or from 1.62 to 8.13 mM for EMPO. Incremental EPR spectra were recorded 30 s after irradiation for 3 min. The initial spin-trapping rate was determined within 120 s by monitoring the first low-field peak intensity of Au@EMPO-CH(CH₃)OH or EMPO-CH(CH₃)OH. All data were the average of three or more measurements.

Decay Kinetics

In a typical kinetic decay study, the sample cell containing the nano spin trap (1 mM) and H_2O_2 (330 μM) in PBS was irradiated for 3 min. The first or second low-field peak decay was monitored as a function of time over a period of 7200 s after light source was turn off. All data were the average of three or more measurements. The reproducibility of peak intensities after 3 min of irradiation is reasonable with a standard deviation of less than 10%.



Equation S1



Scheme S1. The route for the synthesis of Au@PEG3EMPO

Table S1. The atomic concentration in atomic% of the elemental composition.

Sample/%	Au4f	C1s	N1s	S2p	O1s
Au@EMPO	68.83	19.66	5.72	2.44	3.35
Au@PEG3EMPO	8.11	57.81	8.5	5.92	19.66

Table S2. The ICP-MS results of Au@EMPO and Au@PEG3EMPO.

Sample	Au Concentration (mg mL ⁻¹)
Au@EMPO	17.6
Au@PEG3EMPO	15.4

Table S3. The hemolytic activity of Au@EMPO.

Sample	Negative Control	Positive Control	Au@EMPO (µg mL ⁻¹)			
			25	50	75	100
Hemolysis%	0	100	1.2±0.23	1.8±0.21	2.2±0.35	3.1±0.33

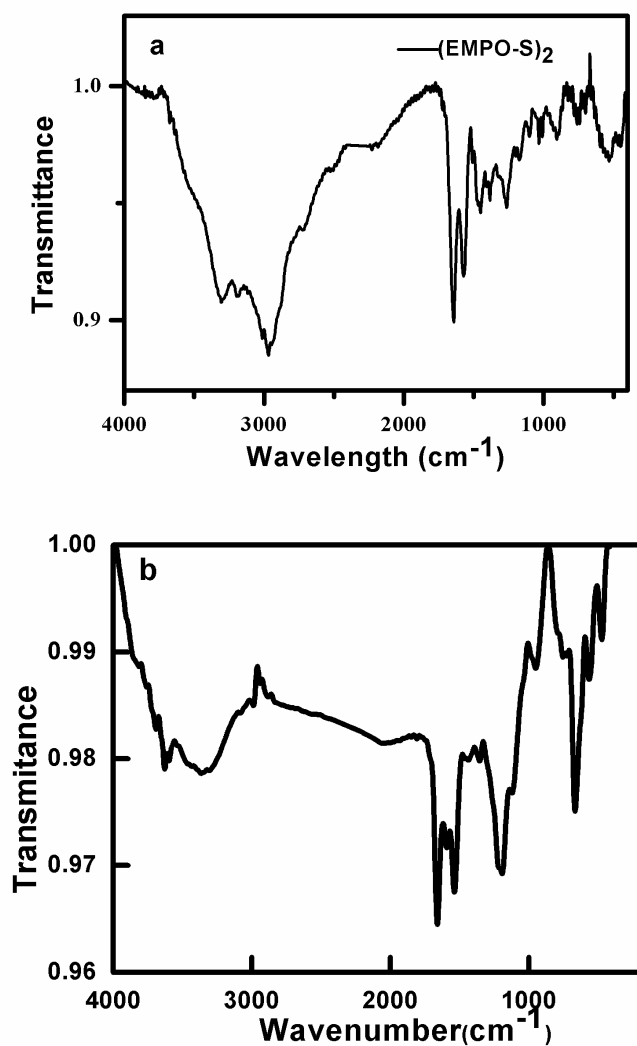


Fig. S1. The FT-IR spectrum of (EMPO-S)₂ and Au@EMPO

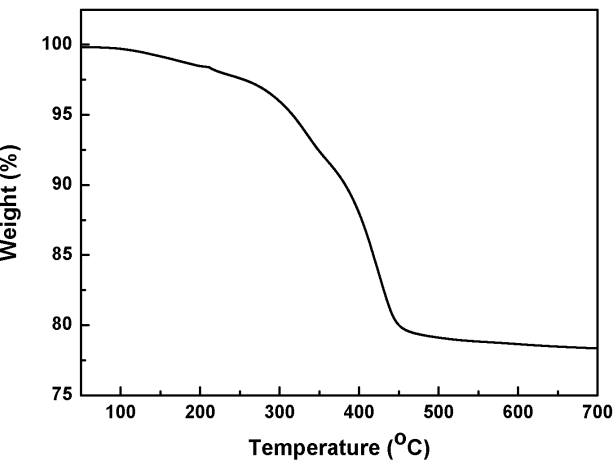


Fig. S2. The TGA results of Au@EMPO.

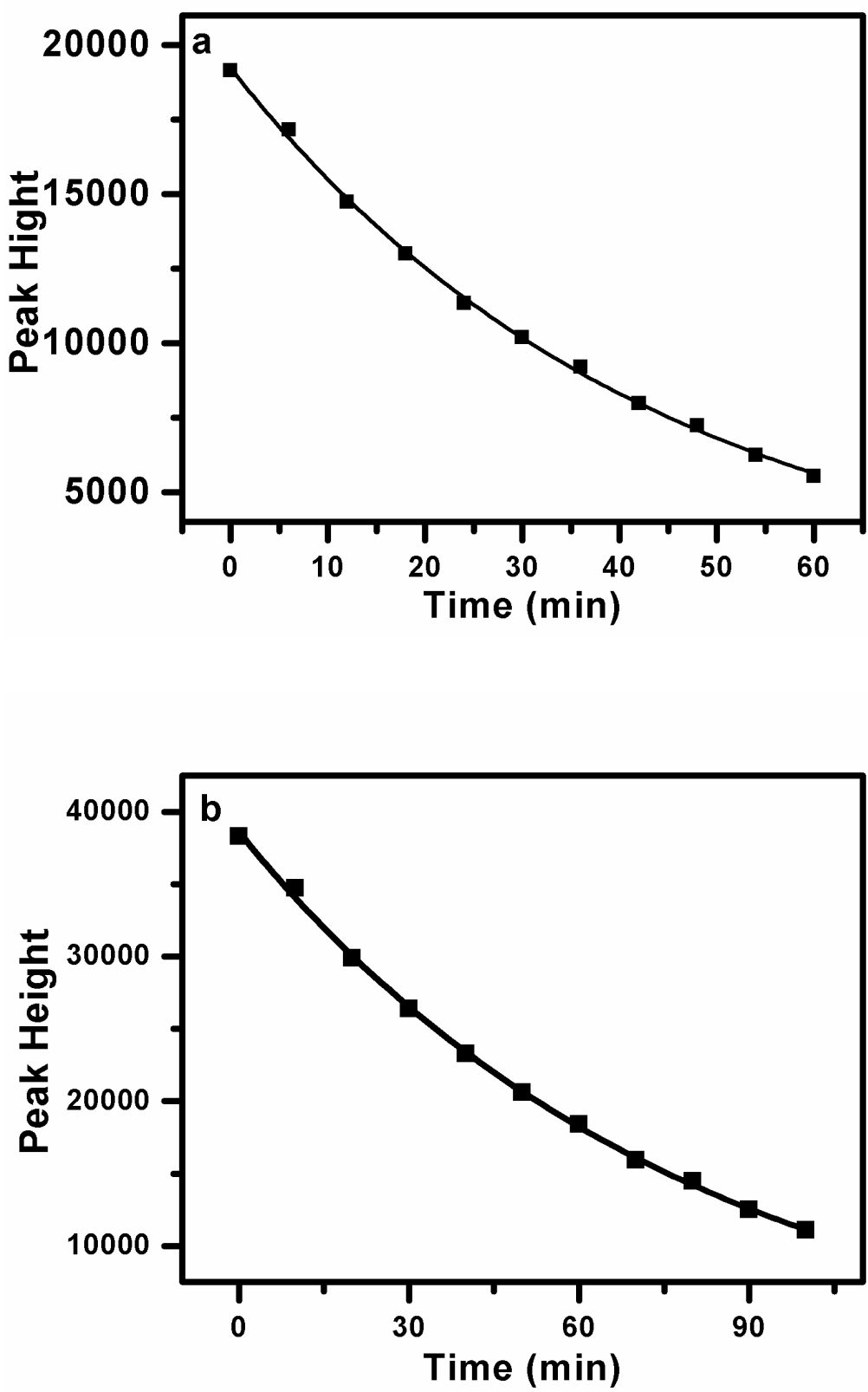


Fig. S3. Kinetics of the decay of the EMPO-OH (a) and Au@EMPO-OH (b) at room temperature.

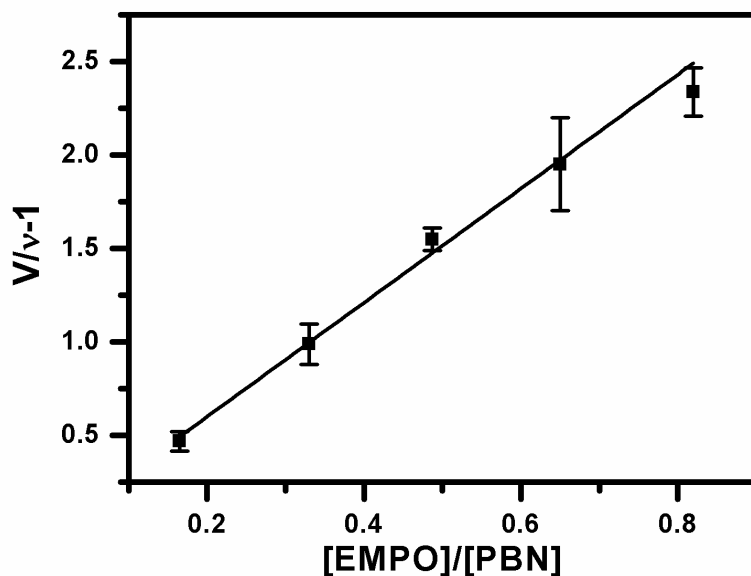


Fig. S4. Typical plot of $V/v-1$ vs $[EMPO]/[PBN]$ using competitive experiments. The $\cdot OH$ competitive trapping of 10 mM PBN with (a) 1.62 mM EMPO , (b) 3.3 mM, EMPO (c) 4.87 mM, EMPO , (d) 6.5 mM Au@EMPO , (e) 8.13 mM, EMPO.

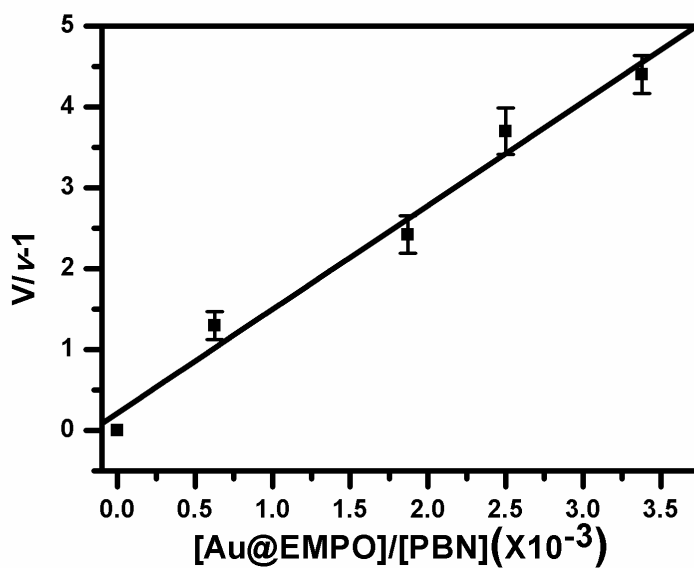


Fig. S5. Typical plot of $V/v-1$ vs $[Au@EMPO]/[PBN]$ using competitive experiments. The $CH_3CH\cdot OH$ competitive trapping of 10 mM PBN with (a) 0 μM Au@EMPO , (b) 6.25 μM , Au@EMPO (c) 18.7 μM Au@EMPO , (d) 25 μM Au@EMPO , (e) 33.8 μM Au@EMPO.

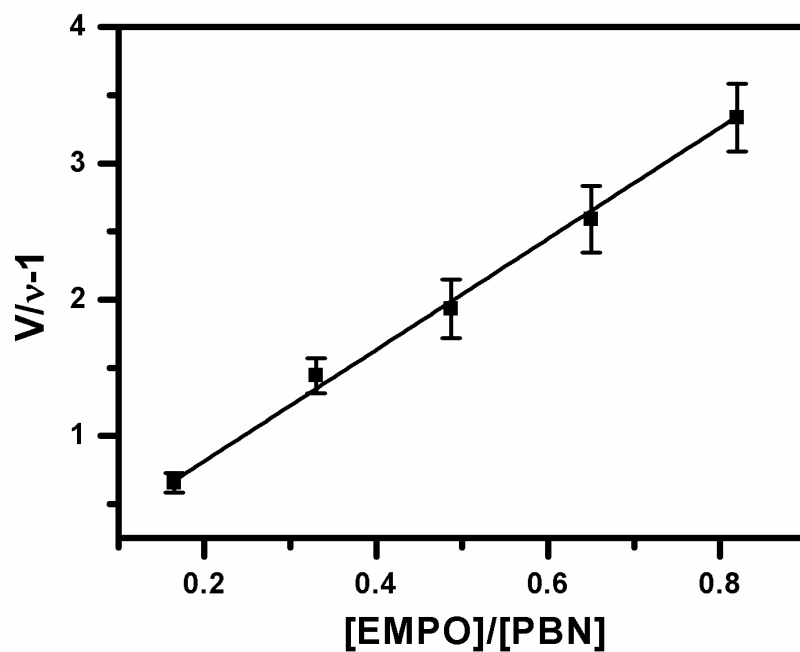


Fig. S6. Typical plot of $V/v-1$ vs $[EMPO]/[PBN]$ using competitive experiments. The $CH_3CH_2\cdot OH$ competitive trapping of 10 mM PBN with (a) 1.62 mM EMPO , (b) 3.3 mM, EMPO (c) 4.87 mM, EMPO , (d) 6.5 mM Au@EMPO , (e) 8.13 mM, EMPO.

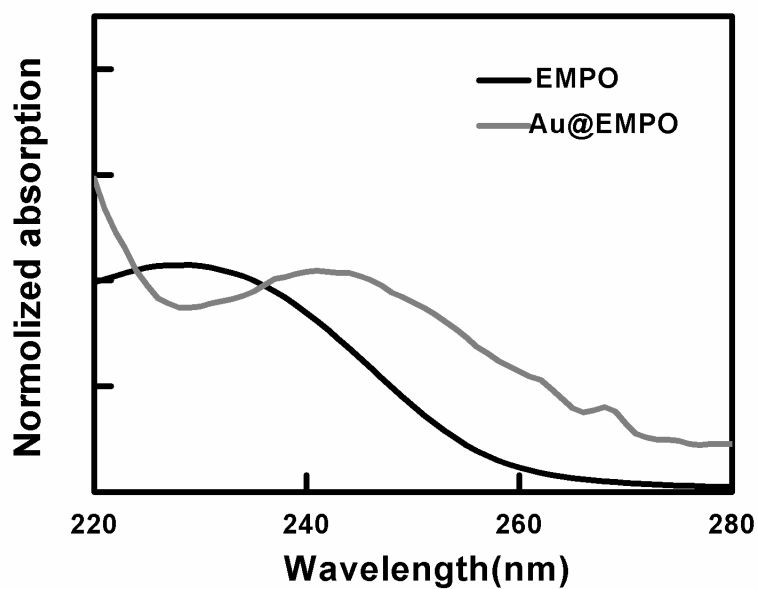


Fig. S7. The red-shift UV-Vis spectra of Au@EMPO and EMPO.

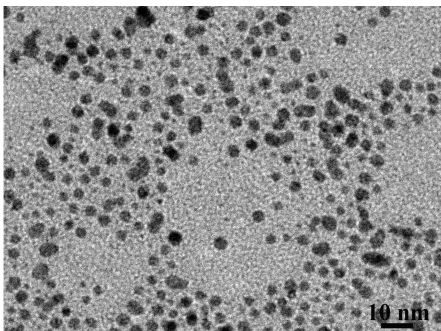


Fig. S8. The TEM image of Au@PEG3EMPO.

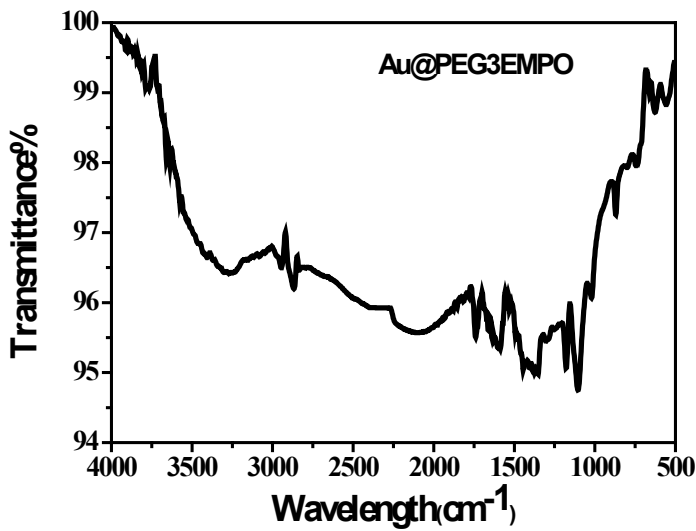
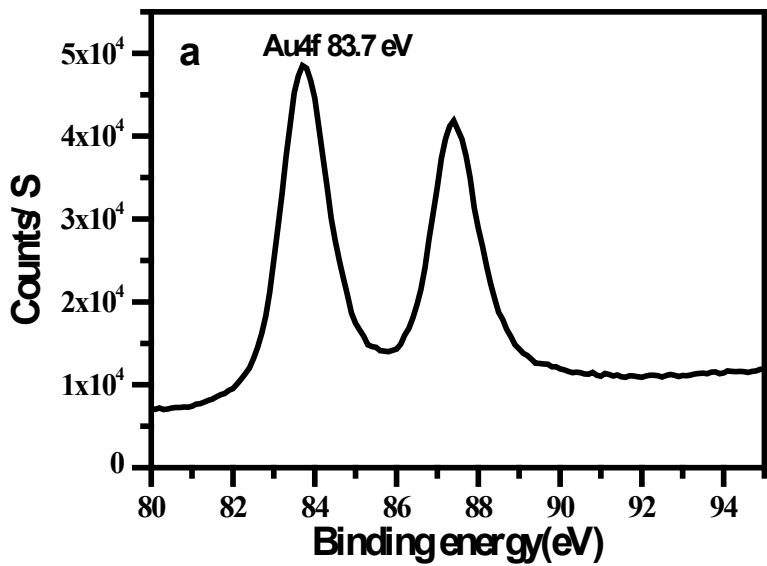


Fig. S9. The FT-IR spectrum of Au@PEG3EMPO.



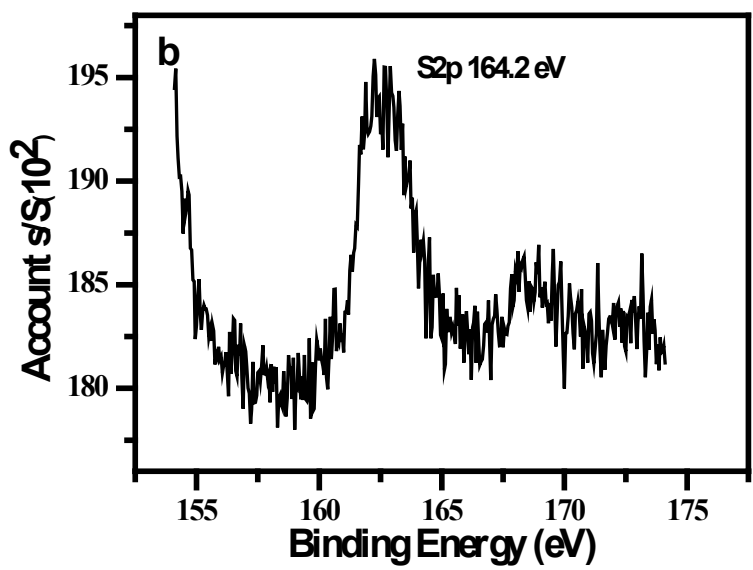


Figure S10. The XPS spectrum of Au@PEG3EMPO. (a) Au4f; (b) S2p.



Fig. S11. The ESR spectrum of Au@PEG3EMPO-OH.

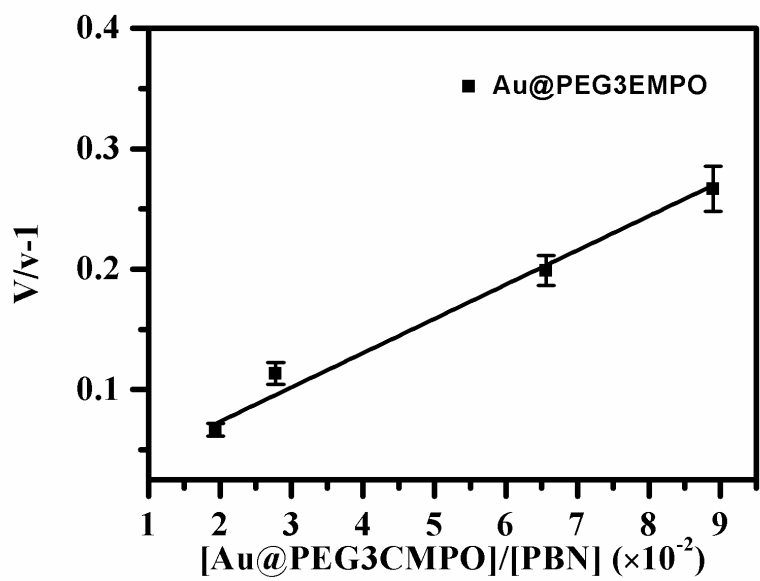


Fig. S12. Typical plot of $V/v-1$ vs $[Au@PEG3EMPO]/[PBN]$ using competitive experiments. The $\cdot OH$ competitive trapping of 10 mM PBN with (a) 6.25 μM , Au@PEG3EMPO, (b) 18.7 μM Au@PEG3EMPO, (c) 25 μM Au@PEG3EMPO, (d) 33.8 μM Au@PEG3EMPO.

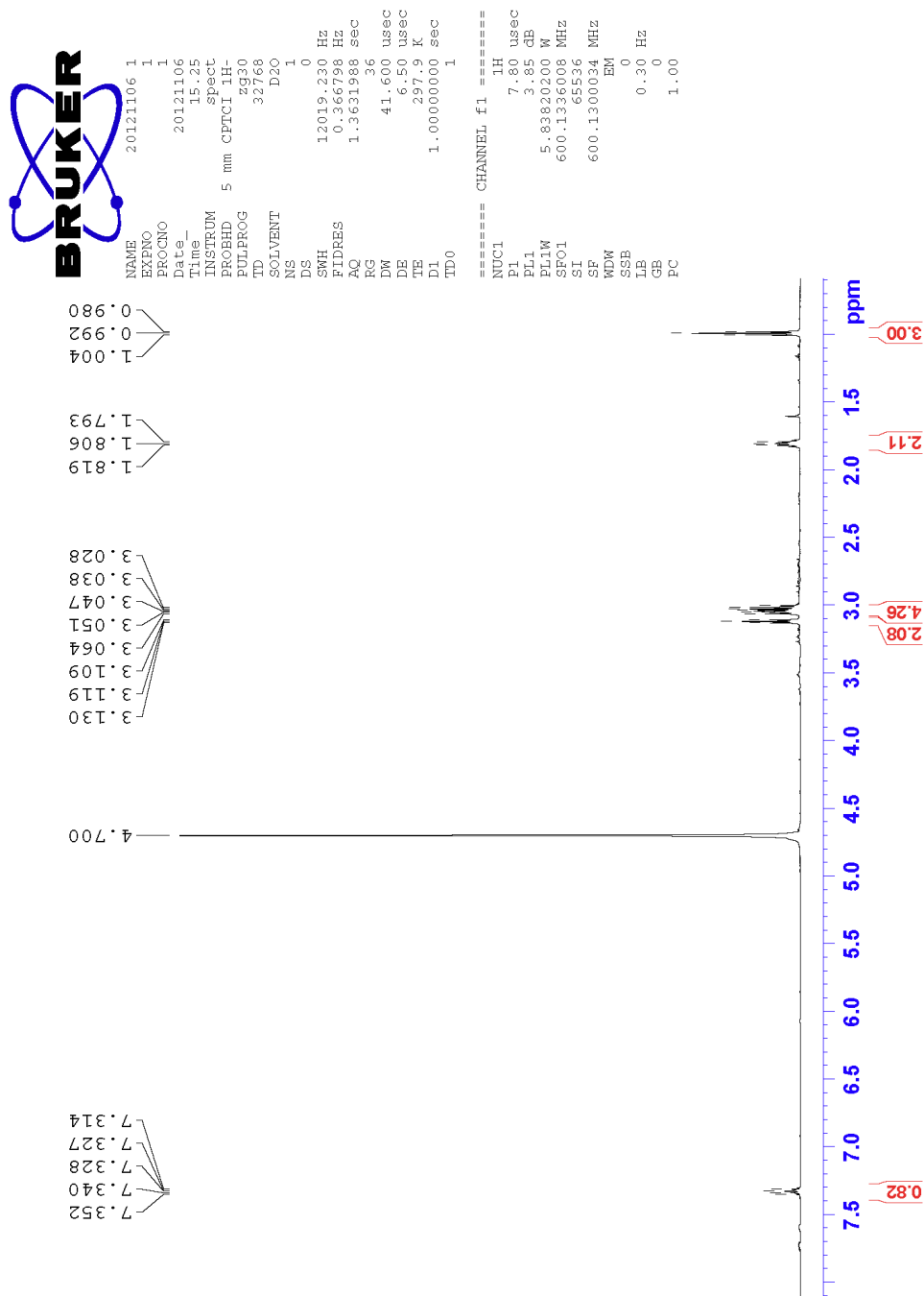


Fig. S13. The 1H NMR of $(EMPO-S)_2$.



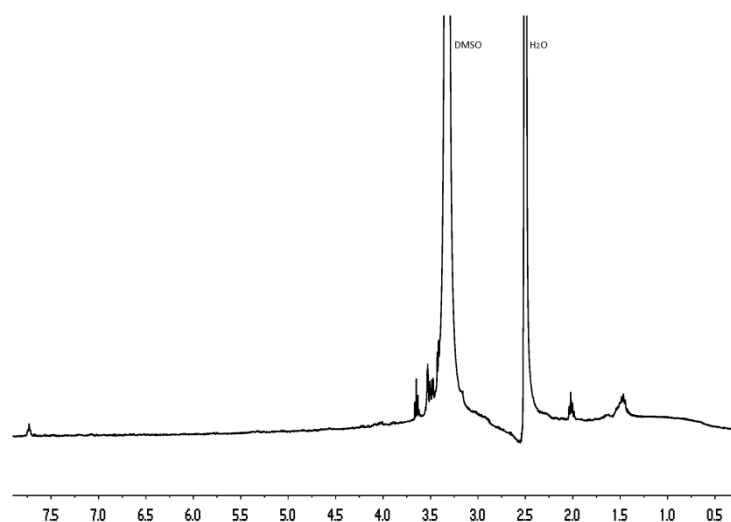


Fig. S15. The ^1H -NMR of Au@EMPO.

References

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