Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Cucurbit[8]uril-based supramolecular nanocapsules with multienzyme-cascade antioxidative effect

Shengda Liu,‡ Ruizhen Tian,‡ Jiayun Xu, Liang Wang, Jianxin Sun, Xiaojia Jiang, Tingting Wang, Xiumei Li, Quan Luo and Junqiu Liu*

State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China

Contents

General Information

Materials

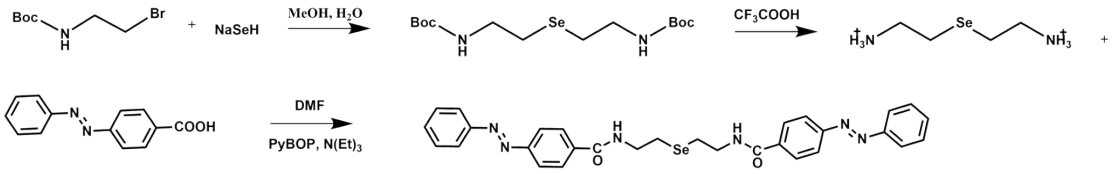
Tert-butyl N-(2-bromoethyl)carbamate, sodium borohydride，4-(phenylazo) benzoic acid, pyrrolidine, 4-pyridinecarboxaldehyde, 1,5-dibromopentane and iodomethane were purchased in Energy Chemical Ltd (Shanghai). Selenium, trifluoroacetic acid (TFA) and 4,4'-bipyridine were purchased from Aladdin Industrial Corporation (Shanghai). RPMI 1640 medium, fetal bovine serum, 24-well plates and 96-well ELISA plates, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Hoechst 33342 staining solution and trypsin were purchased from Meilun Biotechnology Co., Ltd (Dalian). Cucurbit[8]uril (CB[8]), xanthine and xanthine oxidase (XOD) were purchased in Sigma-Aldrich. Other chemical reagents were purchased from Sinopharm Group Ltd (Shanghai).

Instruments and measurements

^{1}H NMR spectra were recorded with a Bruker Avance III 500 instrument using a tetramethylsilane (TMS) proton signal as the internal standard. ESI-MS spectrometric analyses were performed at the Thermo Finnigan-LCQ Advantage Mass Spectrometer. Dynamic light scattering (DLS) measurements were performed at a Malvern Instrument Zetasizer Nano ZS90 instrument. The measurements of UV-vis absorption spectra were recorded on a Shimadzu 3100 UV spectrophotometer. The measurements of fluorescence spectra were performed on a Shimadzu RF-5301 PC spectrofluorimeter. The measurements of atomic force microscopy (AFM) were performed on Nanoscope III a controller, Veeco Metrology, Santa Barbara, CA using tapping modetin with a SiN_4 tip. Transmission electron microscopy (TEM) images were observed by using FEI Talos 200c from a FEI company, operating at the voltage of 120 kV. Scanning electron microscopy (SEM) images were recorded with a JEOL JSM-6700F instrument. Confocal laser scanning microscopy (CLSM) was performed at an Olympus FV1000 instrument. Microplate Reader (ELIASA) was performed at infinite F200 Pro instrument.

Experimental Section

Synthesis of N, N'- (selenobis (ethane-2,1-diyl)) bis (4-((E)-phenyldiazenyl) benzamide) (Se-bisAzo)



Scheme S1. Synthetic route of the Se-bisAzo.

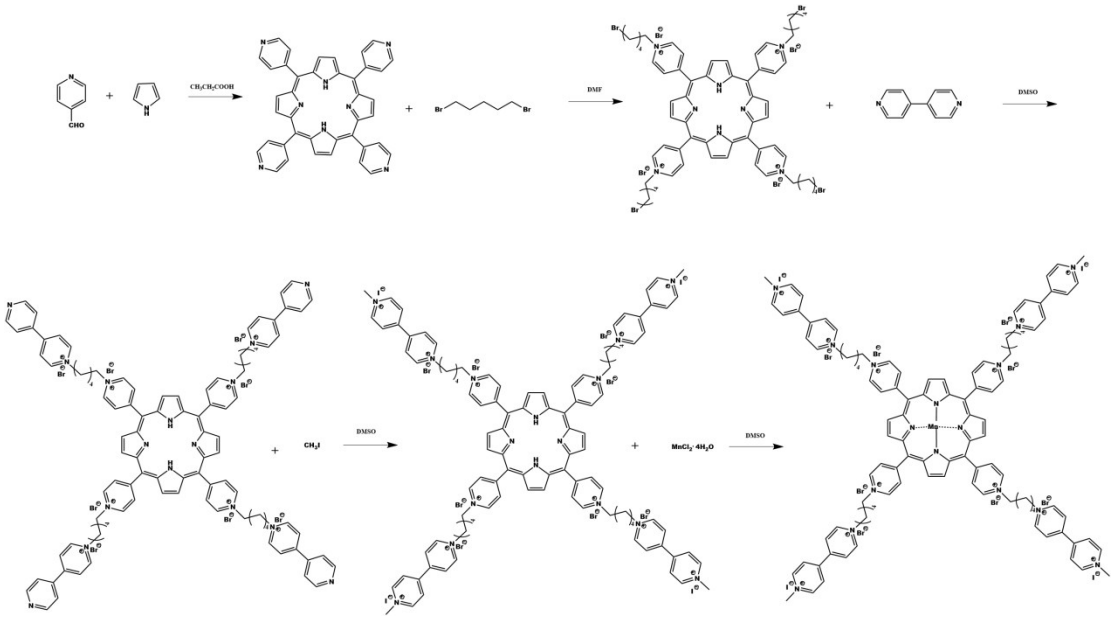
Synthesis of 2, 2'-selenobis(ethan-1-aminium)

The system was completely deoxygenated and in nitrogen atmosphere. Selenium powder (237 mg, 3 mmol) and sodium borohydride (567 mg, 15 mmol) were firstly added to water (50 mL). The mixture was heated and stirred for 30 min to obtain NaSeH. Then MeOH solution of tert-butyl N-(2-bromoethyl)carbamate (1.48 g, 6.6 mmol) was added to NaSeH solution. The mixture was stirred for 24 h at room temperature. Then, the solvent was removed, and the crude product was redissolved in CH_2Cl_2. The organic layer was extracted twice with water and then dried over anhydrous $MgSO_4$. The product of di-tert-butyl (selanediylbis(ethane-2,1-diyl))dicarbamate was purified by silica gel column chromatography. At last, di-tert-butyl (selanediylbis(ethane-2,1-diyl))dicarbamate was added into solution of 10% TFA/$CHCl_3$, the reaction was stirred for 4 h at room temperature. Then, the solvent was removed, and the crude product was purified by recrystallization, yield 65%. ^{1}H NMR (500 MHz, DMSO, 25 ^{o}C) δ (ppm): δ 7.86 (s, 6H), 3.05-3.08 (m, 4H), 2.74-2.77 (m, 4H). ESI-MS: m/z 85.0 ([M] $^{2+}$).

Synthesis of N, N'- (selenobis (ethane-2,1-diyl)) bis (4-((E)-phenyldiazenyl) benzamide) (Se-bisAzo)

Dissolve 2, 2'-selenobis(ethan-1-aminium) (102 mg, 0.6 mmol), 4-(phenylazo)benzoic acid (274 mg, 1.2 mmol) and PyBop (756 mg, 1.44 mmol) in dry DMF (5 mL) and add TEA (1 mL) dropwise. Then the reaction mixture was allowed to proceed at room temperature for 24 hours. The solvent was removed and the product was purified by silica gel column chromatography, yield 78%. ^{1}H NMR (500 MHz, DMSO, 25 ^{o}C) δ (ppm): δ 8.85-8.87 (m, 2H), 8.07 (d, 4H), 7.92-7.97 (m, 8H), 7.61-7.66 (m, 6H), 3.57-3.61 (m, 4H), 2.82-2.85 (m, 4H). ESI-MS: m/z 584.8.

Synthesis of 1,1',1'',1'''- ((manganese-porphyrin-5,10,15,20-tetrayltetrakis(pyridine-1-ium-4,1-diyl))tetrakis(pentane-5,1-diyl))tetrakis(1-methyl-[4, 4'-bipyridine]-1,1'-diium) octabromide tetraiodide (Mn-TMPP)



Scheme S2. Synthetic route of the Mn-TMPP.

Synthesis of 5,10,15,20-tetra(pyridin-4-yl)porphyrin (TPP)

4-Pyridinecarboxaldehyde (3.66 g, 30 mmol) and pyrrole (2.01 g, 30 mmol) were added to propanoic acid (150 mL). The mixture was stirred and refluxed for 3 hours under the condition of nitrogen atmosphere. After cooling to room temperature, the propanoic acid was evaporated by vacuum. The resulting precipitate was washed 3 times with ethanol, yield 23%. ^{1}H NMR (500 MHz, $CDCl_3$, 25 ^{o}C) δ (ppm): δ 9.07 (d, 8H), 8.86 (s, 8H), 8.17 (d, 8H), -2.75 (s, 2H). ESI-MS: m/z 310.1 ([M+2H] $^{2+}$).

Synthesis of 4,4',4'',4'''- (porphyrin-5,10,15,20-tetrayl) tetrakis (1-(5-bromopentyl)pyridin-1-ium) bromide (TBPP)

TPP (1.24g, 2 mmol) and 1,5-dibromopentane (9.12g, 40mmol) in DMF (50 mL) were stirred and refluxed for 24h. After cooling to room temperature, the product was obtained by filtering the reaction mixture and collecting the solid phase. The resulting precipitate was washed 3 times with CH_2Cl_2, yield 25%. ^{1}H NMR (500 MHz, DMSO, 25 ^{o}C) δ (ppm): δ 9.63 (d, 8H), 9.25 (s, 8H), 9.06 (d, 8H), 5.00-5.03 (m, 8H), 3.72-3.74 (m, 8H), 2.33-2.36 (m, 8H), 2.05-2.10 (m,8H), 1.72-1.78 (m, 8H), -3.06 (s, 2H).

Synthesis of 1,1'',1''',1''''- ((porphyrin-5,10,15,20-tetrayltetrakis (pyridine-1-

ium-4,1-diyl))tetrakis(pentane-5,1-diyl))tetrakis(([4,4'-bipyridin]-1-ium)) bromide (TPPP)

TBPP (1.53g, 1mmol) and 4,4'-bipyridine (1.56g , 10 mmol) in DMSO (20mL) were heated to 100℃ and stirred for 24h. After cooling to room temperature, the reaction mixture was poured into precooling CH_2Cl_2 and stayed overnight at -20℃. The product was obtained by filtering the reaction mixture and collecting the solid phase. The resulting precipitate was washed 3 times with CH_2Cl_2, yield 75%. ^{1}H NMR (500 MHz, DMSO, 25 ^{o}C) δ (ppm): δ 9.65 (d, 8H), 9.43 (d, 8H), 9.23 (s, 8H), 9.04 (d, 8H), 8.78 (d, 8H), 8.76 (d, 8H), 8.05 (d, 8H), 5.00-5.03 (m, 8H), 4.85-4.88 (m, 8H), 2.37-2.41 (m, 8H), 2.24-2.28 (m, 8H), 1.65-1.69 (m, 8H), -3.09 (s, 2H).

Synthesis of 1,1',1'',1'''- ((porphyrin-5,10,15,20-tetrayltetrakis (pyridine-1-ium-4,1-diyl))tetrakis(pentane-5,1-diyl))tetrakis(1-methyl-[4,4'-bipyridine]-1,1'-diium) octabromide tetraiodide (TMPP)

TPPP (1.08 g, 0.5 mmol) and iodomethane (710 mg , 5 mmol) in DMSO (20 mL) were stirred at 100℃ for 24h. After cooling down, the reaction mixture was also poured into precooling CH_2Cl_2 and stayed at -20℃ for a night. The product was acquired by filtering the reaction mixture and reserving the solid phase. The resulting precipitate was washed 3 times with CH_2Cl_2, yield 81%. ^{1}H NMR (500 MHz, DMSO, 25 ^{o}C) δ (ppm): δ 9.59 (d, 8H), 9.51 (d, 8H), 9.32 (d, 8H), 9.25 (s, 8H), 9.07 (d, 8H), 8.89 (d, 8H), 8.80 (d, 8H), 4.96-5.00 (m, 8H), 4.84-4.88 (m, 8H), 4.47 (s, 12H), 2.40-2.44 (m, 8H), 2.25-2.29 (m, 8H), 1.72-1.78 (m, 8H), -3.07 (s, 2H).

Synthesis of 1,1',1'',1'''- ((manganese-porphyrin-5,10,15,20-tetrayltetrakis(pyridine-1-ium-4,1-diyl))tetrakis(pentane-5,1-diyl))tetrakis(1-methyl-[4, 4'-bipyridine]-1,1'-diium) octabromide tetraiodide (Mn-TMPP)

TMPP (1.36 g, 0.5 mmol) and $MnCl_2$•$4H_2O$ (990 mg, 5 mmol) were dissolved in DMSO (20 mL) and stirred at 100℃ for 24h under nitrogen atmosphere. After cooling down, the solution was evaporated by vacuum. The product was purified by Sephadex G-25 column chromatography, yield 76%. ESI-MS: m/z 179.4 ([M-4I-6Br] $^{10+}$). The UV absorption peak was shifted from 425 nm (TMPP) to 462 nm (Mn-TMPP).

Preparation of supramolecular nannocapsules

We firstly prepared the DMF solution of Mn-TMPP (10^{-3} M), the DMF solution of Se-bisAzo (2×10^{-3} M), and the aqueous solution of CB[8] (10^{-4} M). Then 100 μL Mn-TMPP DMF solution (10^{-3} M), 100 μL Se-bisAzo DMF solution (2×10^{-3} M) and 4 mL CB[8] aqueous solution (10^{-4} M) were added to the aqueous solution with a total volume of 10 mL in which Mn-TMPP, Se-bisAzo and CB[8] were in a ratio at 1:2:4. The mixtures were treated with ultrasound for 1 h to obtain the supramolecular nanocapsules.

GPx and SOD analysis of supramolecular nannocapsules and the monomers

The GPx activity was determined by the method as described previously.[1,2] The reaction was carried out at 37 ℃ in 500 μL of solution containing 50 mM phosphate buffer (pH = 7.0), 150 μM 3-carboxy-4-nitrobenzenethiol (TNB) and appropriate GPx mimic. The mixture was incubated at 37 ℃ for 1 min, and then 50 μM cumene hydorperoxide (CUOOH) was added to initiate reaction. The activity was determined by measuring the absorbance decrease of TNB at 410 nm using an UV/vis spectrophotometer.

The SOD activity was measured using the standard xanthine/xanthine oxidase (XOD) assay system.[3,4] Superoxide radical anions (O_2-•) were generated by the xanthine/XOD system at 37 ℃ (50 mM phosphate buffer, 0.1 mM EDTA, pH = 7.8). XOD (0.025 U mL^{-1}) was added to 50 mM phosphate buffer containing 0.3 mM xanthine, 0.1 mM NBT, and appropriate enzyme to monitor the oxidation of NBT to blue formazaneat 560 nm using UV/vis spectrophotometer.

Through the above enzyme activity test method, Mn-TMPP showed the SOD activity with the IC_{50} value of 0.407±0.020 μM and Se-bisAzo exhibited the GPx activity of 0.032±0.002 μM min^{-1} (catalyst 100 μM) (Figure S14).

Cell culture

3T3 cells were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The 3T3 cells were incubated with RPMI- 1640 medium containing 10% fetal bovine serum under 5% CO_2 atmosphere at 37°C.

Cell toxicity

The cytotoxicity of the nanocapsules 3T3 cells was determined by MTT assay. 3T3 cells were incubated in 96-well plate for 24 h. The cell medium was removed, and then adherent cells were incubated with nanocapsules (0 to 1 μM). The cells were incubated for another 24 h and treated with MTT for 4 h to generate the insoluble formazan crystal. Finally, the medium was carefully removed and added to the DMSO to dissolve the formazan crystals in each well. Cell viability was quantitatively determined by measuring the absorbance of dissolved formazan at 492 nm.

Intracellular Determination of ROS

The production of ROS was monitored by 2',7'-dichlorofluorescein diacetate (DCFH-DA), a nonfluorescent compound that can react with intracellular free radicals to produce the fluorescent product dichloro-fluorescein (DCF). The intensity of the DCF fluorescence is related to the amount of intracellular reactive oxygen radicals. In experiments, 3T3 cells were cultured in a 6-well plate and a 96-well ELISA plate for 24 h. Then fresh medium containing nanocapsules (0 to 1 μM) replaced old medium. The cells were incubated for another 4 h. Next, the adherent cells were washed three times with PBS (pH 7.4) to remove the excess nanocapsules. The cells were then incubated at 37 °C for 30 min with Rosup (50 μg mL^{-1}). After that, DCFH-DA solution was added to the 3T3 cells, and the mixture was incubated at 37 °C for another 30 min. CLSM image was obtained to determine the 6-well plate at FITC channel at an excitation wavelength 488nm. Fluorescent analysis was performed on ELIASA at an excitation wavelength 485nm and an emission wavelength at 525 nm.

Lipid peroxidation

The lipid peroxidation of mitochondria was assessed by the formation of thiobarbituric acid reactive substances (TBARS) from membrane lipids. The TBARS content in Fe–xanthine/XOD-induced mitochondria was analyzed by thiobarbituric acid (TBA) assay.[5] The procedure was as follows: 1 mL of trichloroacetic acid (70 g L^{-1}) and 1 mL of TBA (5.0 g mL^{-1}) were added into 1 mL of above mixture (mitochondria: 0.5mg L^{-1}) at 80℃ for 1 h. After cooling to room temperature, the mixture was centrifuged at 3000 rpm for 10 min and the supernatant was used to measure the absorption at 532 nm.

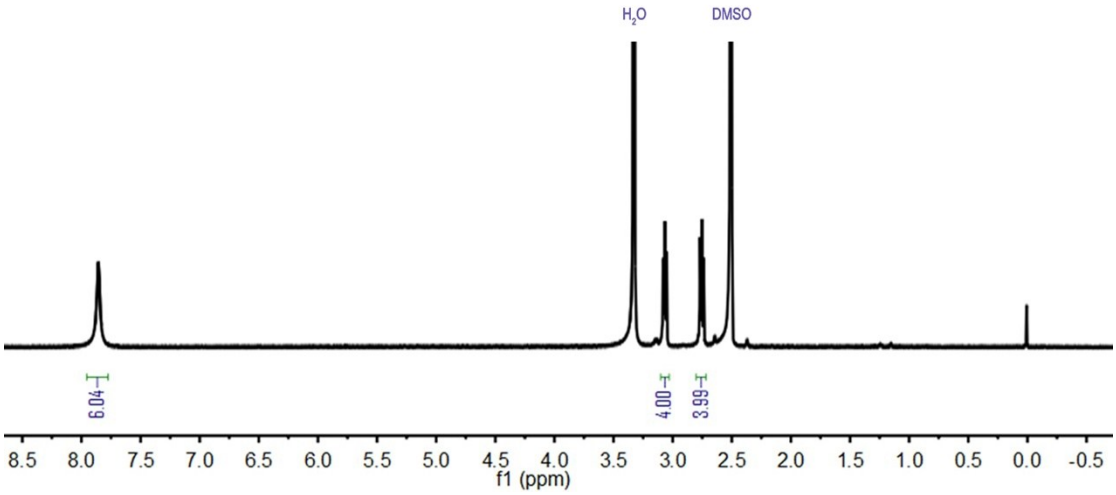


Fig. S1 ^{1}H NMR spectrum of 2, 2'-selenobis(ethan-1-aminium).

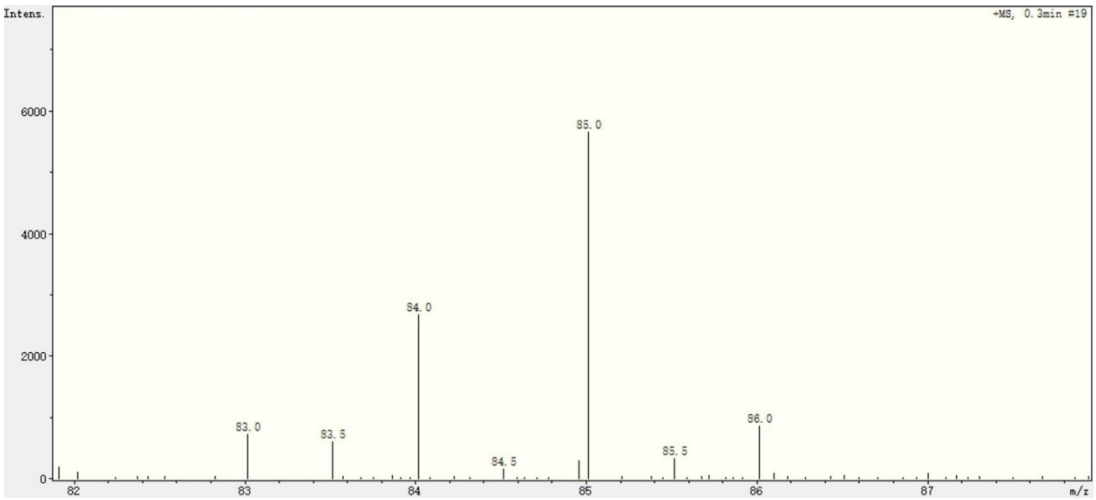


Fig. S2 ESI-MS analysis of 2, 2'-selenobis(ethan-1-aminium).

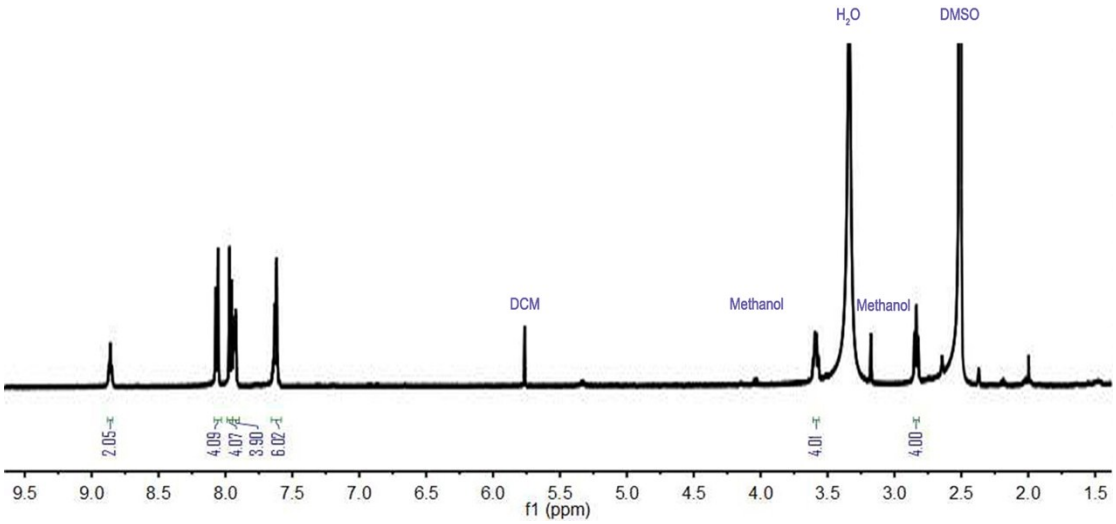


Fig. S3 ^{1}H NMR spectrum of Se-bisAzo.

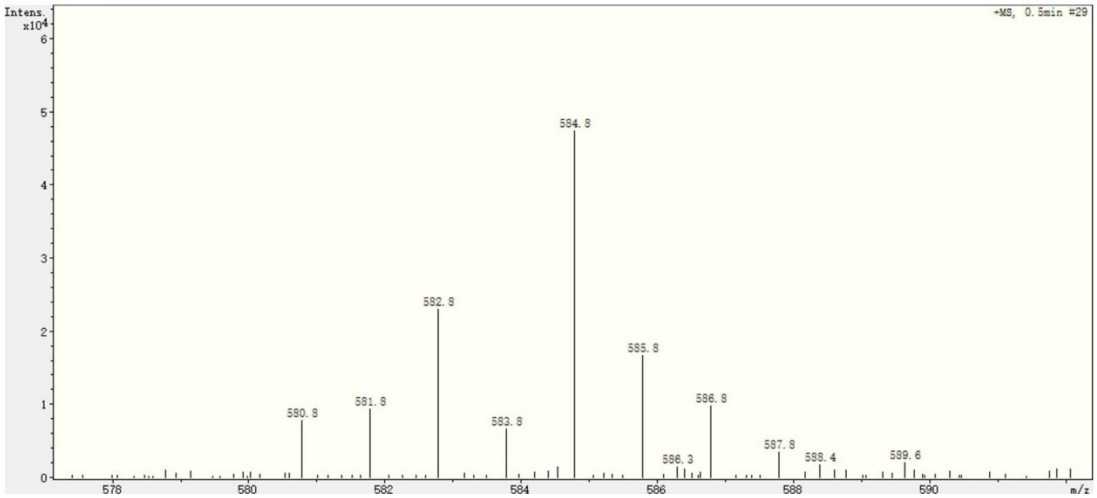


Fig. S4 ESI-MS analysis of Se-bisAzo.

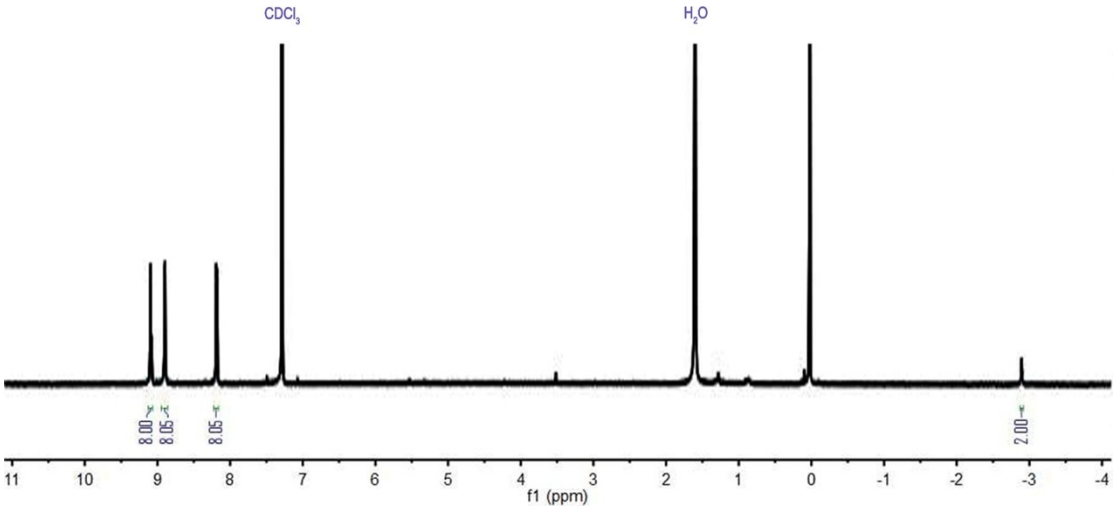


Fig. S5 ^{1}H NMR spectrum of TPP.

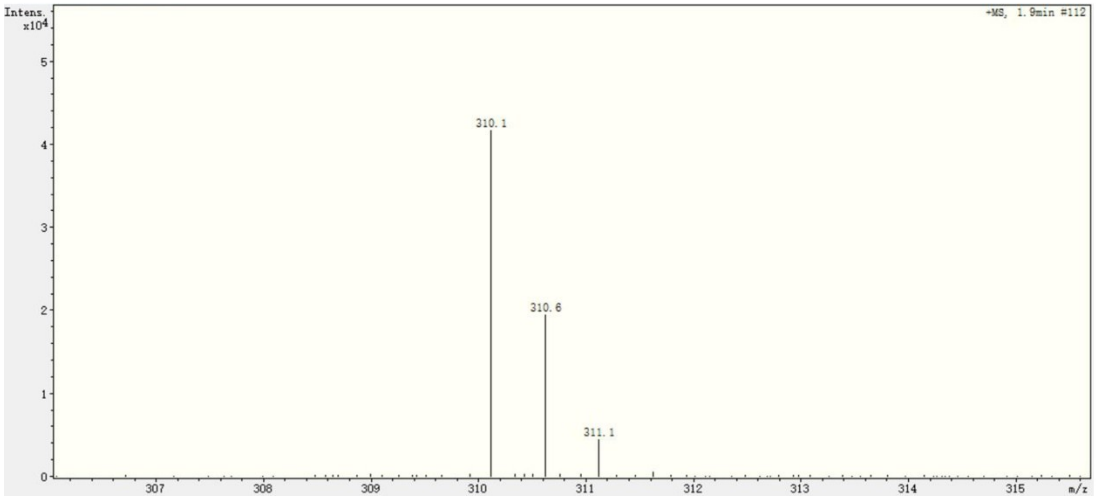


Fig. S6 ESI-MS analysis of TPP.

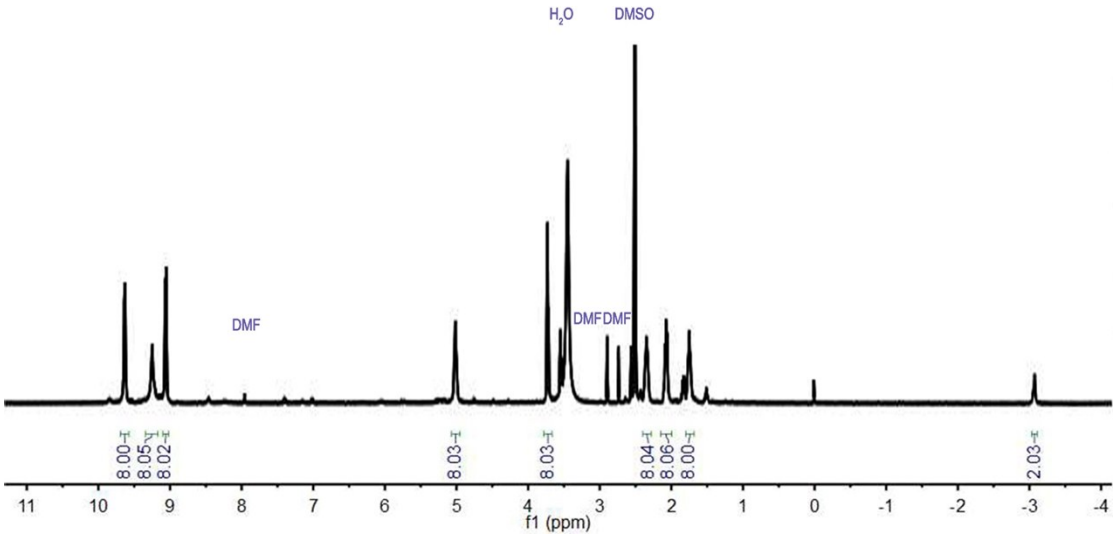


Fig. S7 ^{1}H NMR spectrum of TBPP.

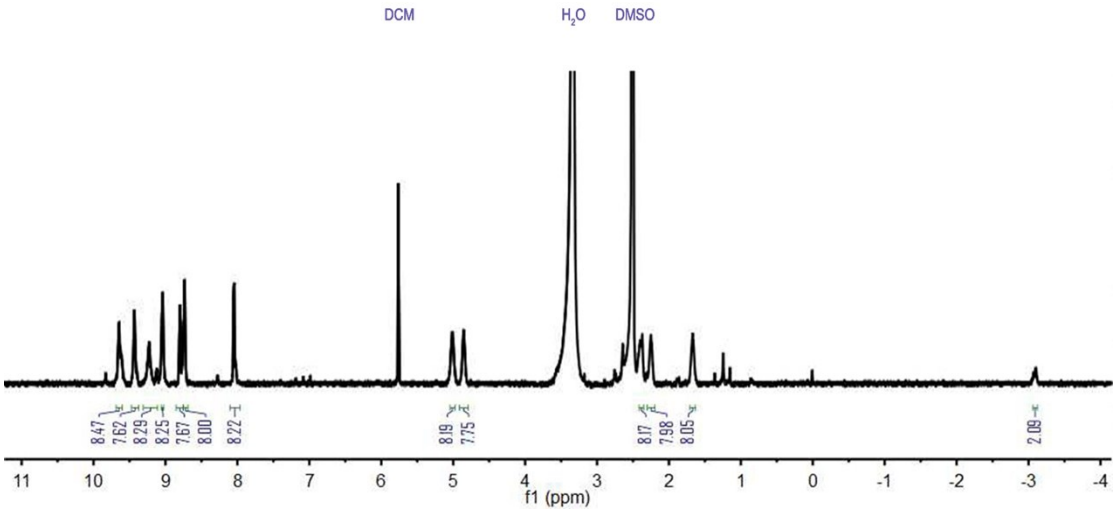


Fig. S8 ^{1}H NMR spectrum of TPPP.

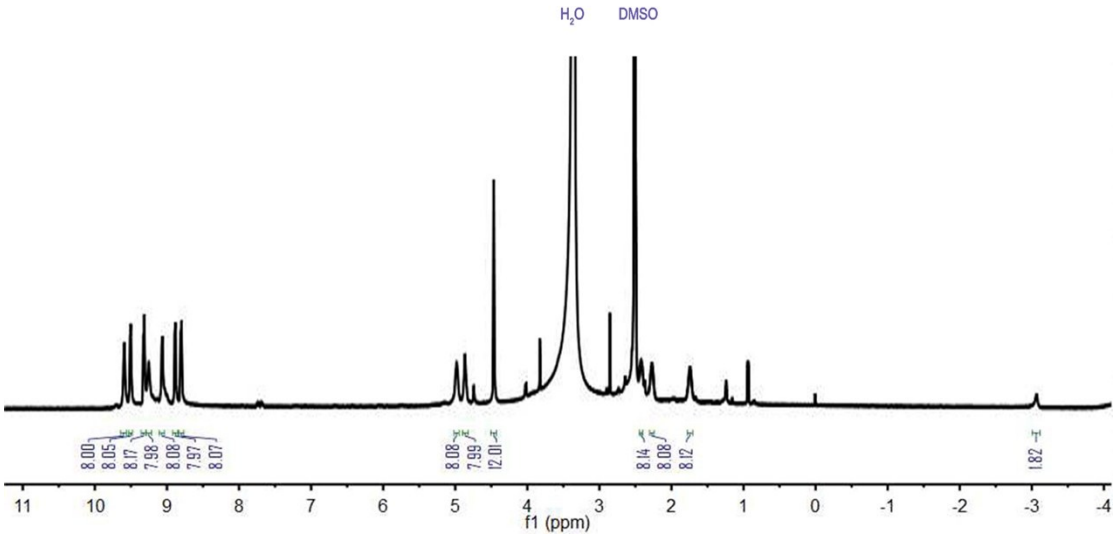


Fig. S9 ^{1}H NMR spectrum of TMPP.

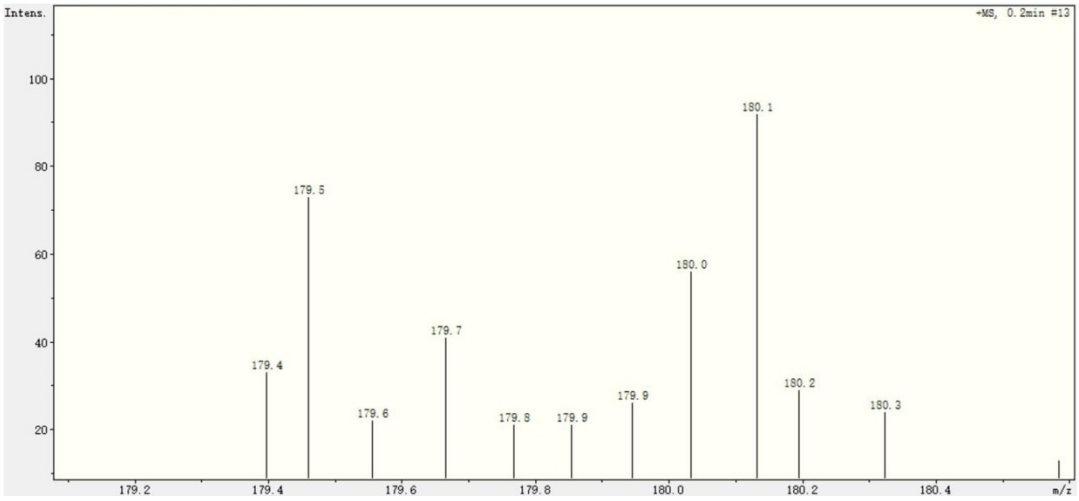


Fig. S10 ESI-MS analysis of Mn-TMPP.

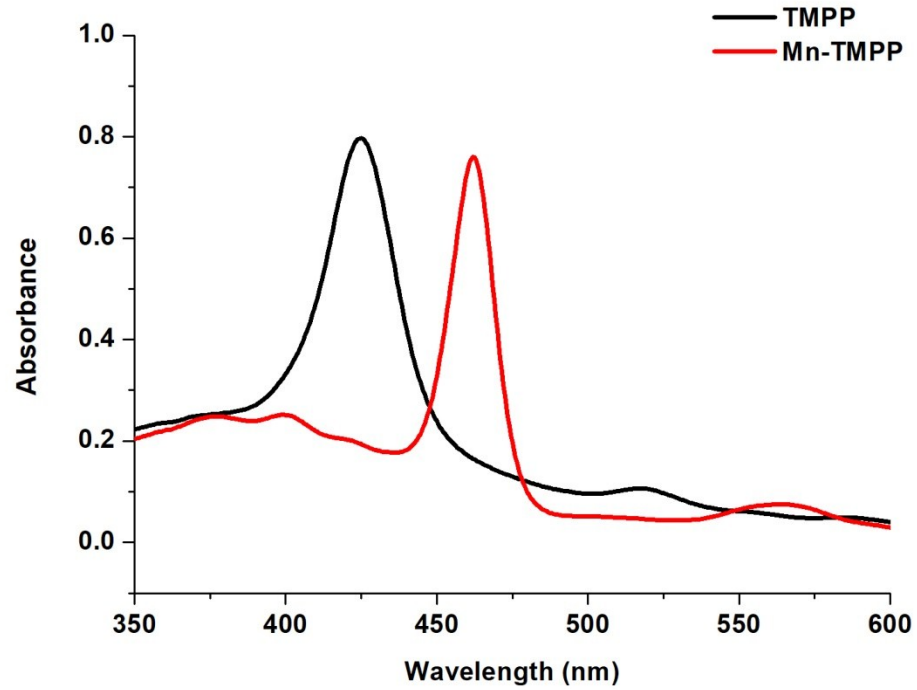


Fig. S11 UV-vis absorption spectra of TMPP and Mn-TMPP.

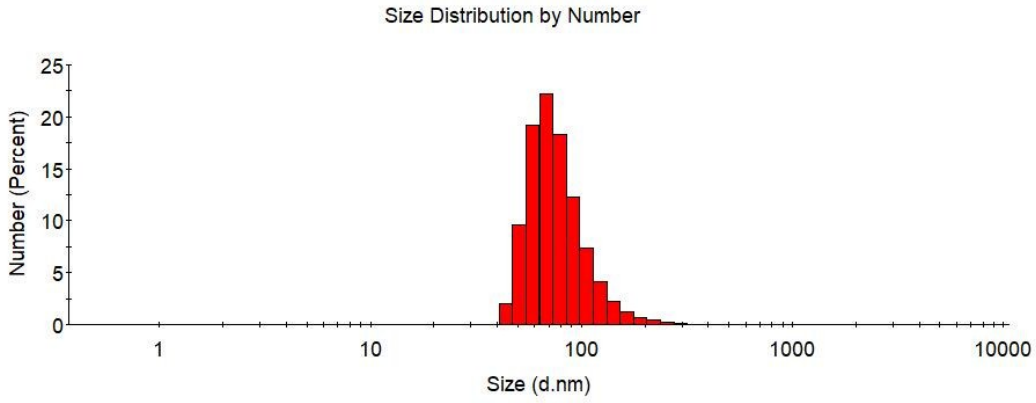


Fig. S12 Hydrodynamic sizes of the supramolecular capsules in aqueous solution.

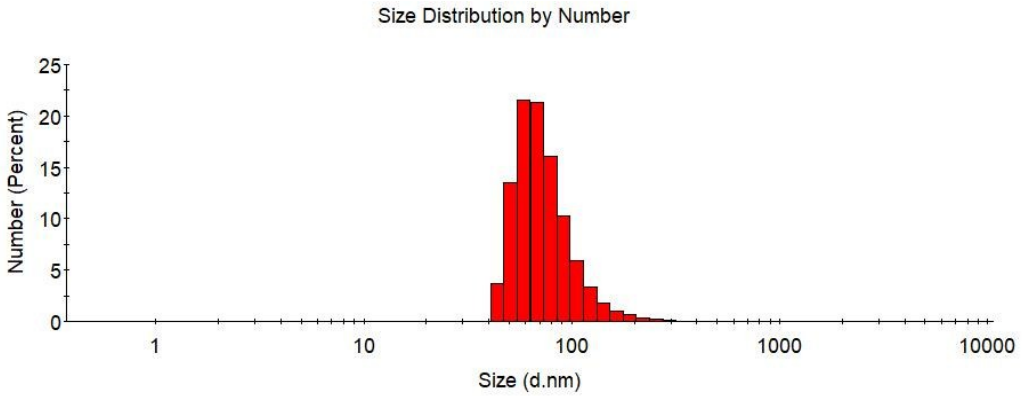


Fig. S13 Hydrodynamic sizes of the supramolecular capsules diluted in PBS solution.

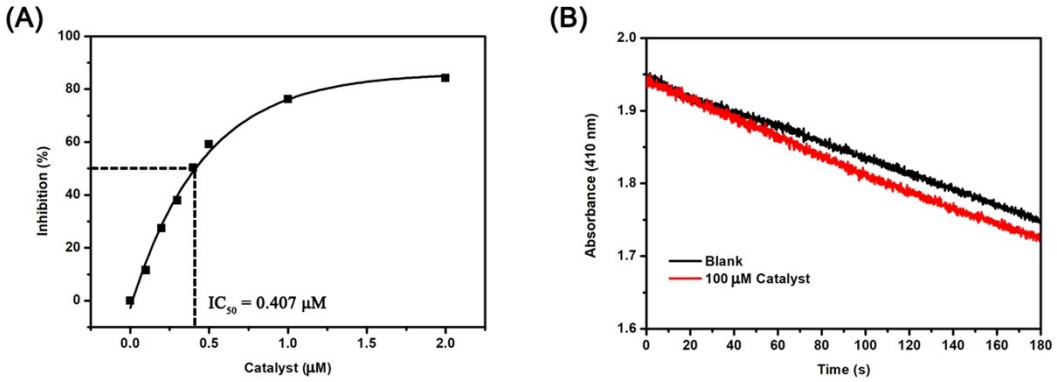


Fig. S14 (A) SOD activity of Mn-TMPP. Percentage of inhibition of NBT oxidation by superoxide anion radical versus different concentrations of Mn-TMPP. (B) GPx activity of Se-bisAzo. Plots of absorbance versus time during the catalytic reduction of CUOOH by TNB.

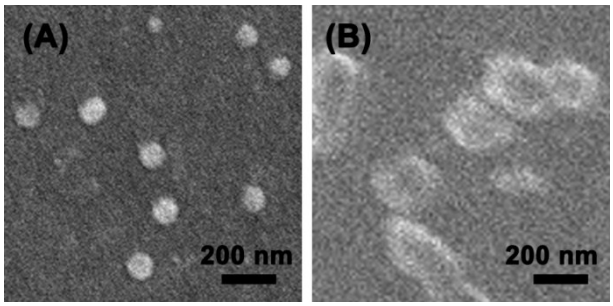


Fig. S15 (A) SEM image of nanocapsules without UV irradiation. (B) SEM image of nanocapsules with UV irradiation.

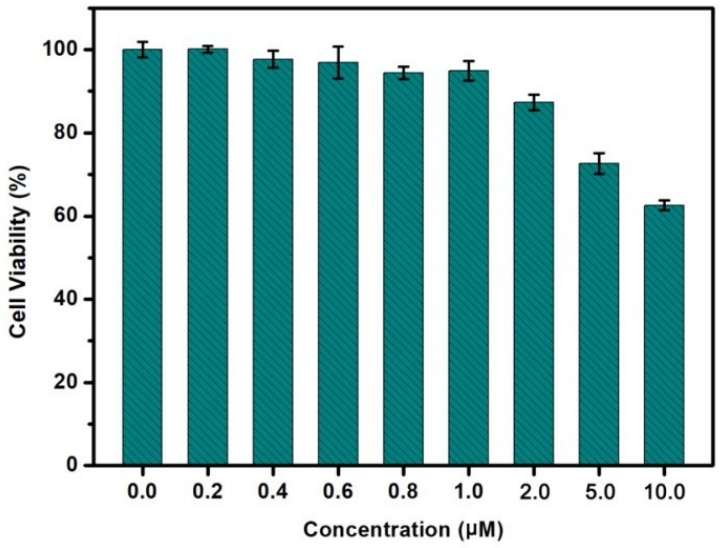


Fig. S16 MTT essay of relative cell viability of the 3T3 cells incubated with different concentrations of Mn-TMPP.

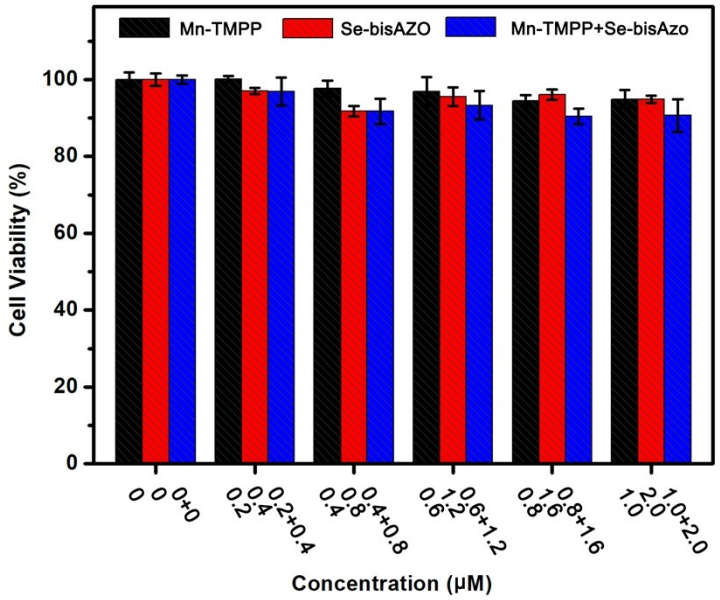


Fig. S17 MTT essay of relative cell viability of the 3T3 cells incubated with different concentrations of Mn-TMPP, Se-bisAzo and Mn-TMPP +Se-bisAzo.

References

1. Y. Yin, S. Jiao, C. Lang and J. Liu, *Soft Matter*, 2014, **10**, 3374–3385.
2. Y. Yin, S. Jiao, C. Lang and J. Liu, *RSC Adv.*, 2014, **4**, 25040–25050.
3. J. M. McCord, I. Fridovich, *J. Biol. Chem.* 1969, **244**, 6049–6055.
4. H. Sun, L. Miao, J. Li, S. Fu, G. An, C. Si, Z. Dong, Q. Luo, S. Yu, J. Xu, J. Liu, *ACS Nano* 2015, **9**, 5461–5469.
5. Y. Gao, C. Hou, L. Zhou, D. Zhang, C. Zhang, L. Miao, L. Wang, Z. Dong, Q. Luo, J. Liu, *Macromol. Biosci.* 2013, **13**, 808–816.