Nitric oxide release triggered by two-photon excited photoluminescence from engineered nanomaterials

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Supplementary Information

Experimental procedure

Materials

Zinc chloride ($ZnCl_2$, 99.999 %), manganese chloride ($MnCl_2$, 99.999 %), 3-mercaptopropionic acid (MPA), hydrazine hydrate ($N_2H_4H_2O$, 80 %), elemental sulfur (99.99 %) and RPMI 1640 cell culture medium were all purchased from Sigma Aldrich Chemical Co. Chitosan (CS, weight-average molecular weight M_w = 108 kDa, degree of acetylation ≥ 90 %) was purchased from Sinopharm Chemical Reagent Co., Ltd, China, and used without further purification. Roussin's Black Salt (RBS, $[NH_4][Fe_4S_3(NO)_7]$) was prepared according to the work of Seyferth et al [1]. The RBS was stored in the dark and inert atmosphere. Ethylene diamine tetraacetic acid (EDTA) was purchased from Aladdin. Fetal bovine serum (FBS) was purchased from Institute of Biochemistry and Cell Biology, CAS. PBS (phosphate buffered saline) buffer was prepared in our own lab. All

raw chemicals were analytical grade unless otherwise stated.

Synthesis of Mn^{2+}-doped ZnS QDs

Mn^{2+}-doped ZnS QDs were synthesized via medium-temperature reaction of the precursors in aqueous phase. In a typical synthetic procedure, 0.64 g of sulfur powder was dissolved in 35 mL of hydrazine hydrate and stirred at room temperature until the sulfur was thoroughly dissolved. The resultant transparent solution was stored for 24 h at room temperature and was diluted 20 times prior to use. 1.6 g of $ZnCl_2$, 0.18 g of $MnCl_2$ and 3.3 g of MPA were dissolved in deionized water, mixed by stirring for 15 min at room temperature. Then 5 mL of the aqueous sulfur-hydrazine hydrate complex was injected and the resultant solution was heated to 50 °C, stirred for 2 h in the open air to produce the Mn^{2+}-doped ZnS QDs. Cooled to room temperature, the QDs were precipitated by the addition of ethanol and by centrifugation at 8000 rpm/min. After thoroughly washing with ethanol and drying in a vacuum oven at room temperature, the QDs were redispersed in water.

Synthesis of Mn^{2+}-ZnS@CS-RBS

5 mg of EDTA was added to 10 mL of as-synthesized Mn^{2+}-doped ZneS QDs aqueous solution and stirred until full dissolution. EDTA facilitated the conjugation of the Mn^{2+}-doped ZnS QDs with CS via chelation. CS was then added into the above solution at a molar ratio to the EDTA of 1 : 2, stirred for 3 min at room temperature. Consequently, ethanol, a nonsolvent for both EDTA and CS, was added dropwise to the solution under stirring until the clear solution turned cloudy, which indicated the formation of Mn^{2+}-ZnS@CS colloidal particles, with the QDs readily

encapsulated in the colloidal particles. The colloidal solution was filtered and centrifuged at 8000 r.p.m for 5 min. The sediment was redispersed into deionized water, dialyzed against deionized water for 12 h to remove the EDTA.

The Roussin's black salt (RBS) was loaded to the as-prepared Mn^{2+}-ZnS@CS via electrostatic interaction. Specifically, 30 mg of Mn^{2+}-ZnS@CS were dispersed in 10 mL of acetic acid aqueous solution under stirring in brown vials. Then 5 mg of Roussin's black salt/ethanol solution was injected and the mixture was stirred for 4 h at room temperature under nitrogen protection. The resultant Mn^{2+}-ZnS@CS-RBS was collected by centrifugation at 8000 r.p.m for 10 min, washed with ethanol, and vacuum dried at room temperature for 3 h.

Characterization

Transmission electron micrographs (TEM) were recorded on a JEM-2100 transmission electron microscope (JEOL, Japan) at 200 kV. Samples were suspended in ethanol, fully dispersed by ultrasonic wave, and deposited on an amorphous carbon coated copper grid prior to observation. The JEM-2100 transmission electron microscope equipped with EDX spectrometry was also used for the energy-dispersive X-ray (EDX) analysis and selected area electron diffraction (SAED). Crystal structure was studied using a D/max-2200/PC X-ray diffractometer (XRD, Rigaku, Japan) fitted with nickel-filtered Cu Kα radiation. Radial scans on the samples were performed from 2θ = 10 ° to 60 °. The data were collected at 0.02° intervals with counting for 0.2 s at each step. The size distribution of the samples were determined by a Nano ZS90 particle size and zeta potential analyzer (Malvern, UK) based on dynamic light scattering (DLS) at a scattering angle of 90°. The Mn doping level was determined by inductively coupled plasma-atomic emission spectroscopy

(ICP-AES, Leeman, US). The dopant concentration obtained from the ICP-AES was 1.65 at%. Single-photon-absorption and two-photon-absorption induced PL and PL excitation spectra were collected with a Fluorolog-3 fluorescence spectrophotometer (Horiba Jovin Yvon, France) equipped with an external 0-10 W adjustable continuous wave laser (300-1600 nm) tuned at 1160 nm as the excitation source. The incident laser pulses were focused by a 10 cm focal length lens onto a 1 cm-thick quartz cell containing the samples. PL emission lifetime was measured on the Fluorolog-3 fluorescence spectrophotometer at 25 °C. The sample was excited using a laser source at 1160 nm with a pulse duration of 5 μs. Electron spin resonance (ESR) experiments were operated at liquid nitrogen temperature on an ESP 300 EPR spectrometer (Bruker, Germany) with the microwave frequency of 9.22 GHz and the microwave power of 10 mW. The sweep rate was 100 G/min.

Determining two-photon absorption cross-section of Mn^{2+}-ZnS@CS-RBS

The two-photon absorption cross-section of Mn^{2+}-ZnS@CS-RBS was obtained from the ratio of the measured PL from Rhodamine 6G (two-photon absorption cross-section of 10 GM) to the Mn^{2+}-ZnS@CS-RBS:

$$(F_{Rh} / F_{sample}) = [(\eta\sigma)_{Rh} \cdot \rho_{Rh} \cdot I_{00}^2] / [(\eta\sigma)_{sample} \cdot \rho_{sample} \cdot I_{00}^2] \quad (1)$$

where F is the PL intensity, η is the PL quantum yield, σ is the two-photon absorption cross-section, ρ is the concentration, and I_{00} is the peak intensity on the beam propagation axis. $(\eta\sigma)_{Rh}$ is known and F_{Rh} is measured at 1160 nm.

Determining PL quantum yield of Mn^{2+}-ZnS@CS-RBS

The PL quantum yield (QY) of the as-prepared Mn^{2+}-ZnS@CS-RBS was measured relative to Rhodamine 6G in water. (QY = 94 %) [2]. The absorption spectra of Mn^{2+}-ZnS@CS-RBS and Rhodamine 6G aqueous solutions at different concentrations were recorded. Then the PL spectra of both Mn^{2+}-ZnS@CS-RBS and Rhodamine 6G were recorded under the same conditions. The PL quantum yield was calculated according to the following equation:

$$\phi_{Sample} = \phi_{R6G} * \left(\frac{F_{Sample}}{F_{R6G}}\right) * \left(\frac{A_{R6G}}{A_{Sample}}\right) * \left(\frac{n_{Sample}}{n_{R6G}}\right)^2 \quad (2)$$

Where ϕ_{Sample}, F_{Sample}, A_{Sample} and n_{Sample} are the quantum yield, integrated PL intensity, integrated absorption and refractive index of the solvent for the Mn^{2+}-ZnS@CS-RBS. The parameters with a subscript of RG6 are corresponding quantities of the solvent for Rhodamine 6G.

Measurement of NO release from Mn^{2+}-ZnS@CS-RBS

Quantitative detection of the NO molecules released from the Mn^{2+}-ZnS@CS-RBS was carried out by a TBR 4100/1025 free radical analyzer equipped with an ISO-NOP sensor (WPI Ltd., US). The dynamic detection range of the NO sensor is 0.3 nM to 100 μM. The light-triggered NO release was measured at 37 °C, where the detection sensitivity was determined to be 2.55 pA/nM. The details are as follows: 0.1 mg of the nanospheres was dispersed in 1 mL of a 0.2 M PBS buffer (pH = 7.4), forming a stable suspension. Then the suspension was rapidly injected into 19 mL of the PBS buffer when the ISO-NOP sensor had reached a low, stable current level. The NO probe was immersed about 2 cm into the suspension under magnetic stirring. The sensor was accurately calibrated by mixing different volumes (100, 200, 400, 800, 1600 μL) of 50 μM KNO_2 solution with 0.1 M KI and 0.1 M H_2SO_4 solutions, producing NO according to the following

reaction [1]:

$4H^+ + 2I^- + 2NO_2^- \rightarrow 2H_2O + 2NO + I_2$

Cell assay

L929 cells (mouse fibroblast cells) purchased from Institute of Biochemistry and Cell Biology, CAS, were cultured in RPMI 1640 medium supplemented with 10 wt% FBS, 100 IU/mL penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin in a humidified incubator with 5 vol% carbon dioxide at 37 °C. The medium was refreshed every 2 or 3 days according to cell density. Cytotoxicity of the Mn^{2+}-ZnS@CS-RBS was evaluated by 3-(4,5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium chloride (MTT) viability assay. The L929 cells were seeded in 96-well culture plates at a density of 4500 cells per well and incubated at 37 °C for 24 h for cell attachment. The culture medium in each well was then replaced by a fresh medium containing the Mn^{2+}-ZnS@CS-RBS at different concentrations (0.01 mg/mL - 1 mg/mL). One row of the 96-well plates was used as control. After further incubation for 24 h or 48 h, the culture plates were rinsed with a PBS buffer (0.01 M, pH = 7.4) to remove unattached cells and the remaining cells were treated with 5 mg/mL MTT stock solution in PBS for 4 h. The medium containing unreacted MTT was then carefully removed. The obtained formazan was dissolved in DMSO, and the absorbance of individual wells was recorded at 570 nm using a Multiskan MK3 Enzyme-labeled Instrument (Thermo Scientific, US). The cell survival rate was determined by the following equation:

$$\text{cell survival rate \%} = \text{absorbance of test cells/absorbance of control cells} \times 100\ \% \quad (1)$$

In vitro Cell imaging

A suspension of L929 cells incubated with 0.1 mg/mL Mn^{2+}-ZnS@CS-RBS for 4 h was transferred to an eight-well Lab-Tek II chamber slide (Nalge Nunc, Napevillem, IL). The medium was then aspirated from the wells, and were rinsed with fresh culture medium for three times. The cell fluorescence excited by two-photon absorption was observed by a confocal laser scanning microscope (Zeiss LSM 710, Germany) equipped with an NIR II excitation source. The excitation was provided by a 1160 nm laser diode and the emitted photons were collected using a 550 nm long-pass filter.

References

1. X. Zhang, *Front. Biosci.*, 2004, **9**, 3434-3446.

Supplementary data

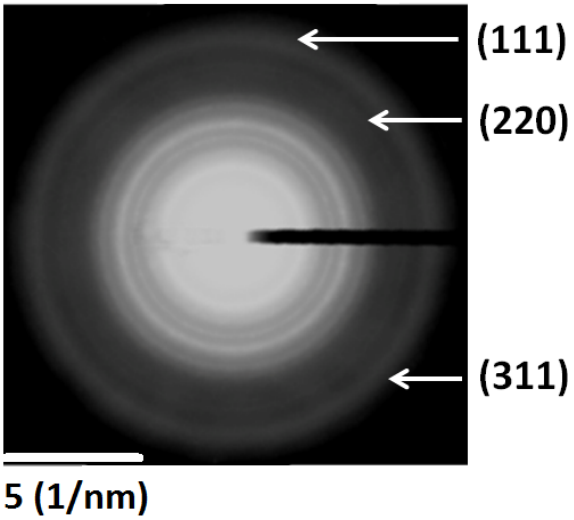

Fig. S1 SAED pattern of Mn^{2+}-doped ZnS QDs with the Miller indexes indicated.

size distribution of as-synthesized Mn^{2+}-doped ZnS QDs

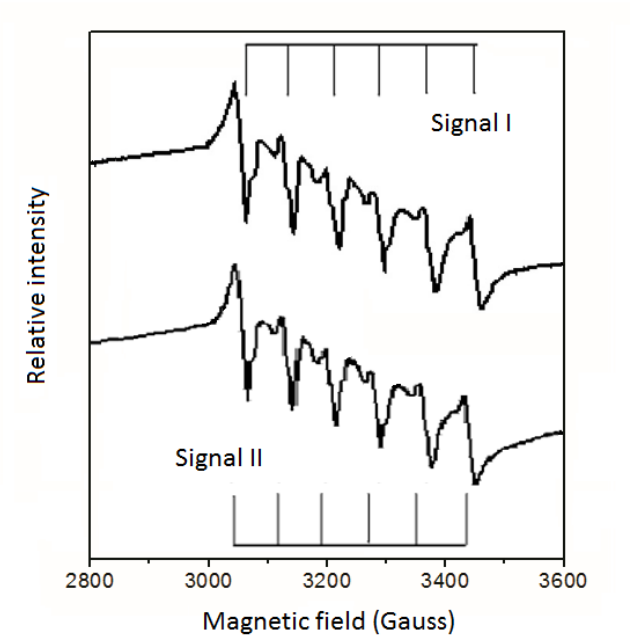

Fig. S2 ESR spectra of Mn^{2+}-doped ZnS QDs and ii Mn^{2+}-ZnS@CS-RBS. Signal I arises from substitutional incorporation of Mn^{2+} ions in ZnS QDs; signal II arises from Mn^{2+} ions located near the QD suface.

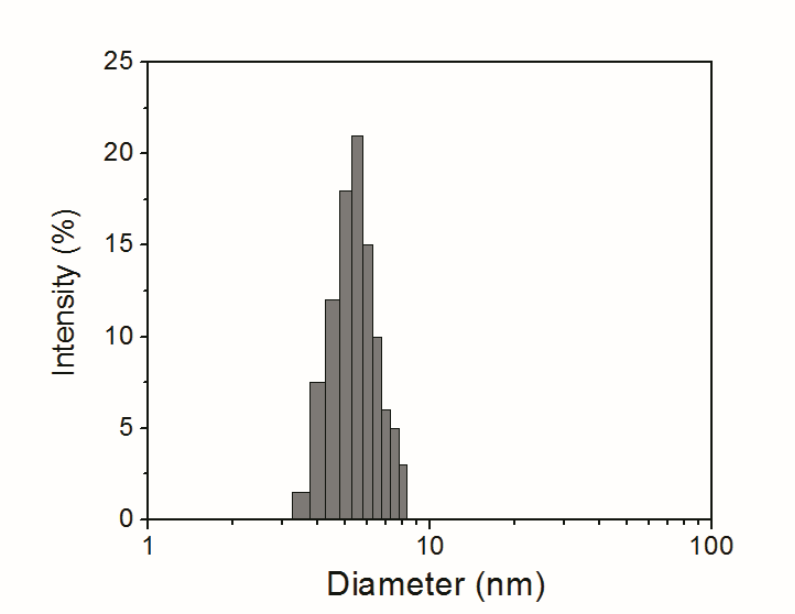

Fig. S3 Size distribution of the Mn^{2+}-doped ZnS QDs based on DLS test.

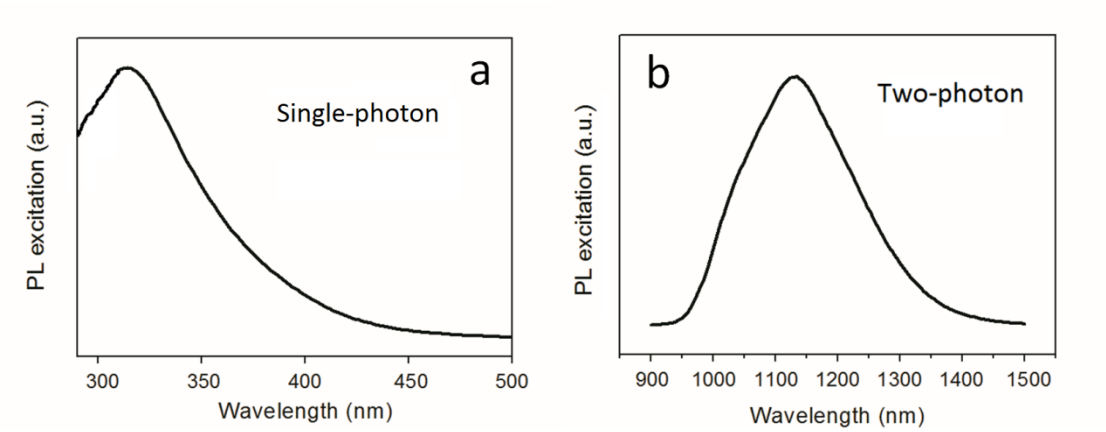

Fig. S4 Single-photon (a) and Two-photon (b) PL excitation spectra of Mn^{2+}-ZnS@CS-RBS.

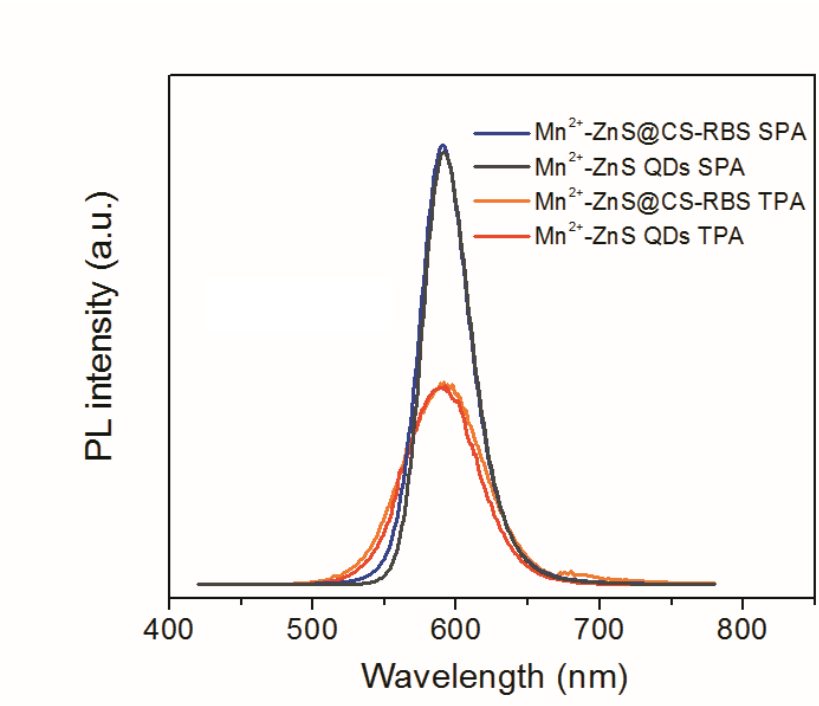

Fig. S5 Single-photon and two-photon excited PL emission spectra of Mn^{2+}-doped ZnS QDs and Mn^{2+}-ZnS@CS-RBS. The excitation wavelength was 314 nm for SPA and 1160 nm for TPA.

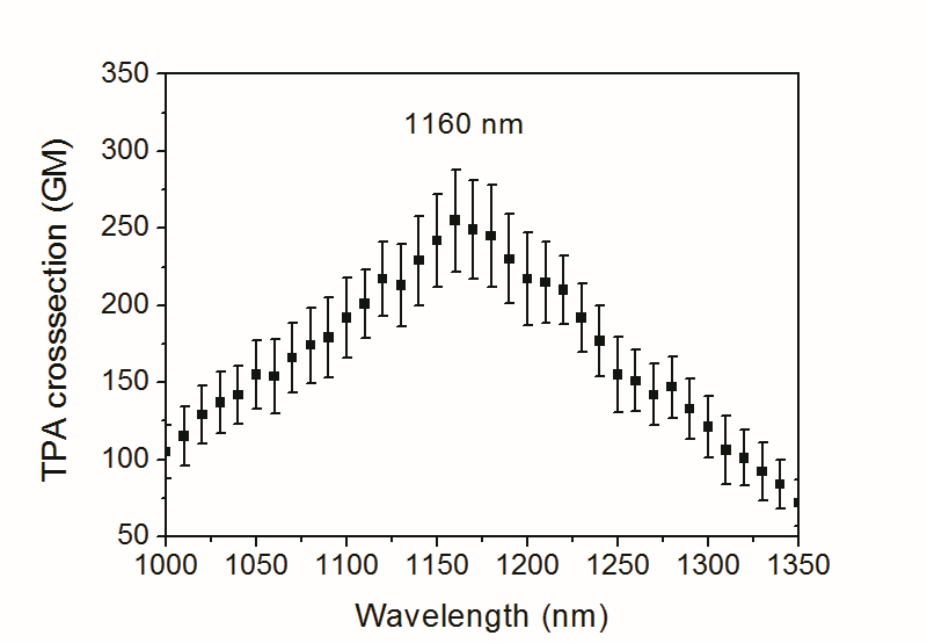

Fig. S6 Two-photon absorption cross-section estimated for Mn^{2+}-ZnS@CS-RBS in the range from 1000 to 1350 nm.

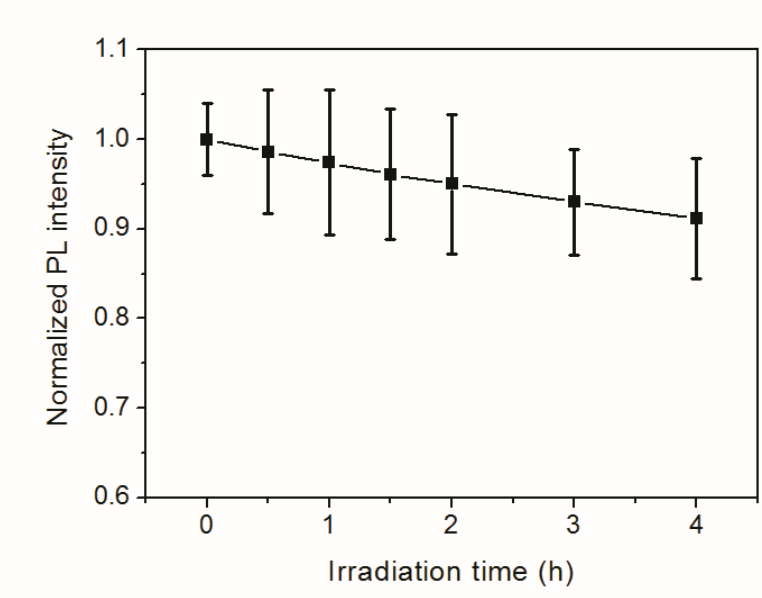

Fig. S7 PL intensity changes of Mn^{2+}-ZnS@CS-RBS with time under irradiation of 1160 nm laser. The irradiation power output was 100 W.

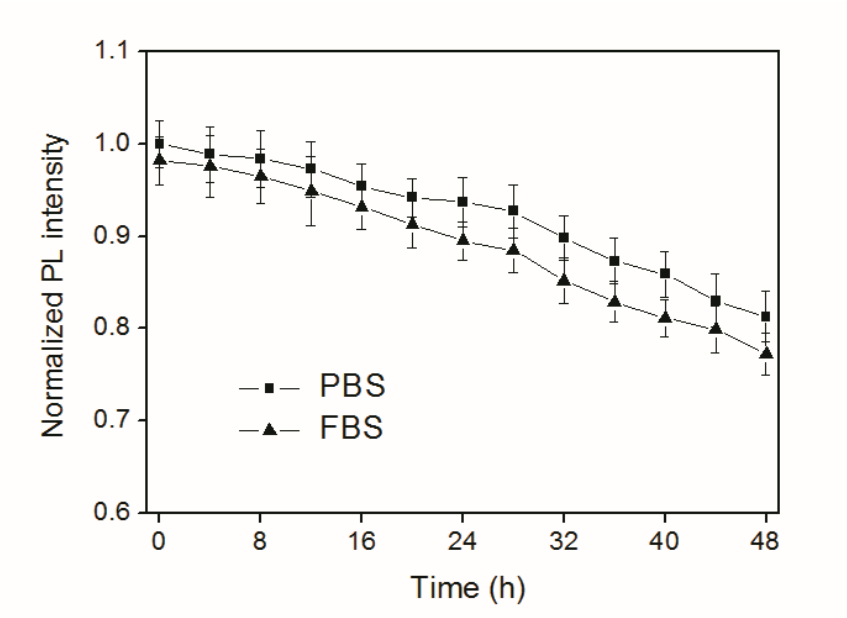

Fig. S8 Relative PL intensity of Mn^{2+}-ZnS@CS-RBS in PBS buffer and 10 % FBS solution over a period of 48 h.

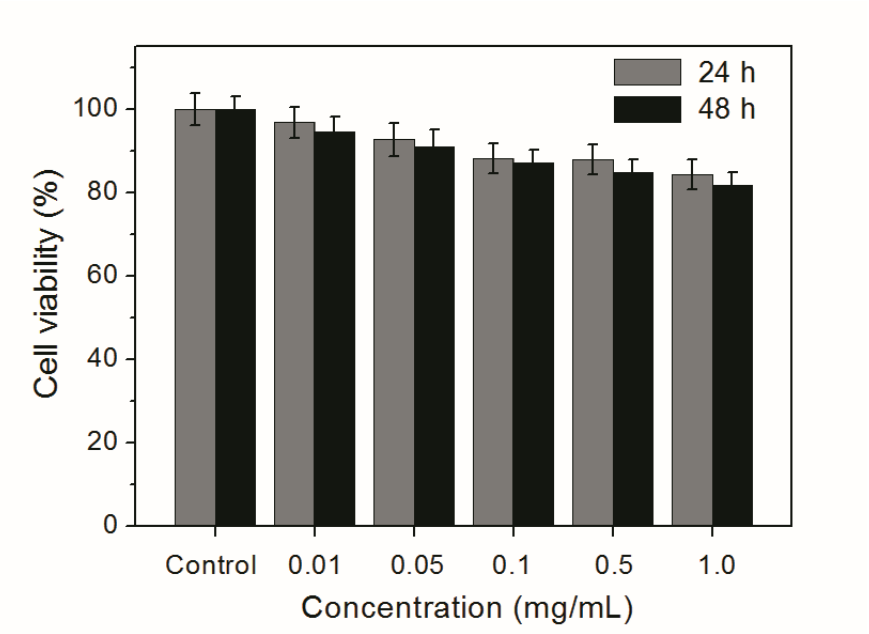

Fig. S9 Viability of L929 cells incubated with different concentrations of Mn^{2+}-ZnS@CS-RBS for 24 h and 48 h. Error bars represent SD (n=8).

Table 1 Influences of irradiation power (λ=1160 nm) on the .NO releasing properties of Mn^{2+}-

ZnS@CS-RBS.

Irradiation power (W)	Duration time (min)	Total NO release (μM)
2	145	4.1
4	136	6.2
6	120	8.6
8	107	10.1