Electronic Supplementary Information

Fluorescent Se-modified Carbon Nitride Nanosheets as Biomimetic catalase for Free-Radical Scavenging

Xu-Ning Cao, Shu Lian, Yawen Tong, Wei Lin, Lee Jia, Yuanxing Fang and Xinchen Wang*

Experimental Section

***Synthesis of CN*:** CN was synthesized according previous literature. Melamine (1.5g) was calcined at 550 ℃ for 4h in air condition with a heating rate of 250 ℃ h^{-1}. The obtained yellow powder was called CN.

Synthesis of bulk SeCN: The bulk selenium doped carbon nitride material was prepared by thermal polymerization of trichloromelamine (TCMA) and potassium selenocyanate (SeCN) with the moto rate (1:1). First, TCMA (3.43 g) was grounded with SeCN (2.16g) in fume hood. Then, the mixture was heated to 550 ℃ for 4 h under a N_2 protection (2 $Lmin^{-1}$) in a muffle furnace. The obtained solid samples were grinded again and washed with deionized water to remove the ions. The final samples were dried in vacuum oven at 60 ℃ overnight.

***Synthesis of SeCNNS*:** The SeCNNS was prepared by liquid exfoliation of bulk SeCN. First, the as-prepared bulk SeCN was dispersed in deionized water with the concentration of 1g/ml. Then the samples were sonicated for 16 hours. The initial formed suspension was then centrifuged at 10000 rpm to remove the aggregates before used for further study.

Characterization: The samples were characterized X-ray photoelectron spectroscopy (XPS) with Thermo ESCALAB250 instrument with a monochromatized Al Kα line source (200 W).

The Fourier transformed infrared (FTIR) spectra were recorded on BioRad FTS 6000 spectrometer. The powder X-ray diffraction (XRD) measurements were obtained on Bruker D8 Advance diffractometer with Cu Ka1 radiation (k = 1.5406 Å). The photoluminescence (PL) spectra was performed by fluorescence spectrophotometer (F-7000, Hitachi, Varian Ltd., USA). The electron paramagnetic resonance (EPR) results were performed by Bruker Model A300 spectrometer. The UV-Vis diffuse reflectance spectra (DRS) of the catalysts were obtained on a Varian Cary 500 Scan UV-visible system, using $BaSO_4$ as a reflectance standard. The absorption spectra were measured by UV-2700 (Shimadzu Ltd, Japan). The morphology of the sample was investigated by field emission scanning electron microscopy (SEM) was operated by Nova Nano 230 microscope. (JSM-6700F) and transmission electron microscopy (TEM) images were performed by a FEI TECNAL G2F20 microscope. The atomic force microscopy (AFM) images were tested by system Multimode 8 (Bruker Ltd, USA). Zeta potentials were measured using a Malven Zetasizer Nano ZS90.

DFT Calculations:

All the calculations were performed based on density functional theory (DFT) via the Vienna ab initio simulation package (VASP)[1]. The exchange−correlation functional were treated described by the generalized gradient approximation (GGA)[2] of the Perdew−Burke−Ernzerhof (PBE)[3] form. The DFT-D2 method was chosen to consider the van der Waals dispersion correction[4]. A cut-off energy 520 eV for plane-wave basis set were applied. The structural configurations were fully optimized until the energy change is less than 10^{-4} eV and force change is less than 0.01 eV Å-1. Supercell (1 x 1x 1) was repeated periodically on the x-y plane, and the vacuum thickness was set to 10 Å along the z-direction

in order to avoid interactions between adjacent slabs. The Brillouin zone (BZ) was sampled by 1 × 2 × 1 Monkhorst−Pack mesh k-point grid[5].

In vitro cell assays:

The A549 cells were purchased from the Cell Resource Center of Shanghai Institute for Biological Sciences (Chinese Academy of Sciences, Shanghai, China). The A549 cells were cultured in 1640 RPMI medium containing 10% FBS and 1% penicillin-streptomycin (v/v) and maintained in a humidified cell incubator (Galaxy 170S, Germany) at 37 °C with 5% CO_2.

Cytotoxicity assay:

Cytotoxicity of SeCNNS and H_2O_2 were studied by typically MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) method. The 10,000 cells were seeded into 96-well plates for per well. After 24 h, medium with SeCNNS in different concentrations or different percent H_2O_2 were added in the plates. After continued incubation for another 24 h or 1 hour, the cytotoxicity effect for different complexes was assessed by the MTT assay. The absorbance at 490 nm was determined by a microplate reader (TECAN M200 PRO, Switzerland). Normal cultured cells served as control group. All samples were repeated 5 times. Besides, cytotoxicity of different concentration of SeCNNS suspensions with 0.078% H_2O_2 or 0.05% were tested by MTT in the same way.

Flow cytometer ROS test:

ROS detection is through the DCFH-DA kit (ROS fluorescent probe, Beyotime Biotechnology Ltd, China). Briefly, 50,000 A549 cells were seeded in each 6-well plates. After 12 hours, medium with different SeCNNS concentrations or/and 0.01%,0.015% H_2O_2

was added to each well. After incubated another 1 hour, cells was digested by 0.25% trypsin and washed twice by PBS. And then the cells were dyed by 5 μM DCFH-DA for 20 min at 37 ℃. Finally, A549 cells were washed twice by PBS and analyzed by Flow cytometry (BD FACS AriaIII, USA) at 488 nm excitation.

C***onfocal experiment:***

Firstly, 50,000 A549 cells were seeded in each confocal dish. After 12 hours, medium with 50 ug/mL SeCNNS was added to dish. After incubation with the SeCNNS for another 4 hours, and washed twice with PBS, SeCNNS material fluorescence was detected by confocal microscopy (Leica TCS SP8, Germany) 405 nm excitation.

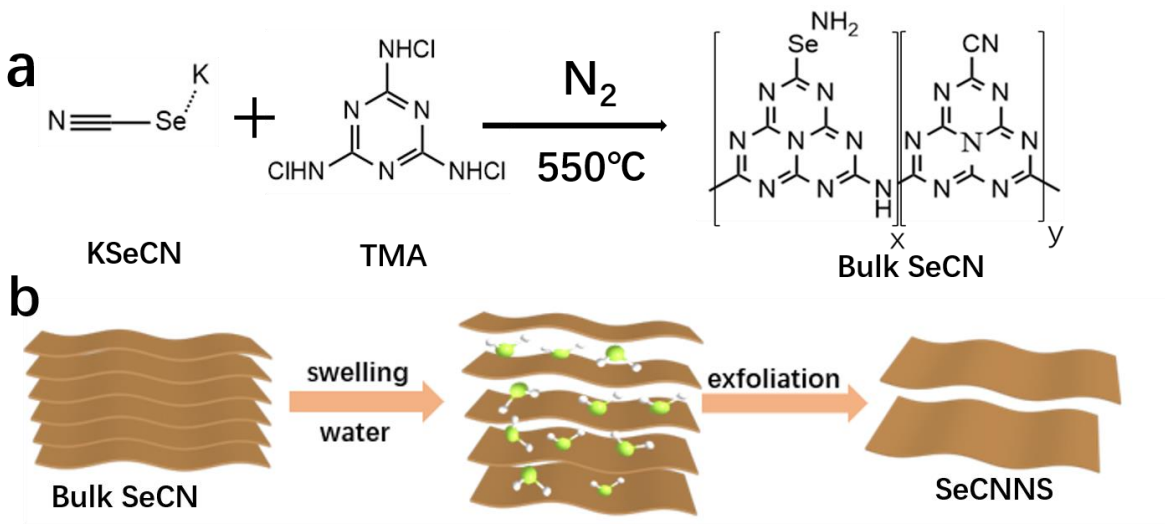

Fig. S1. Synthesis procedure of SeCNNS.

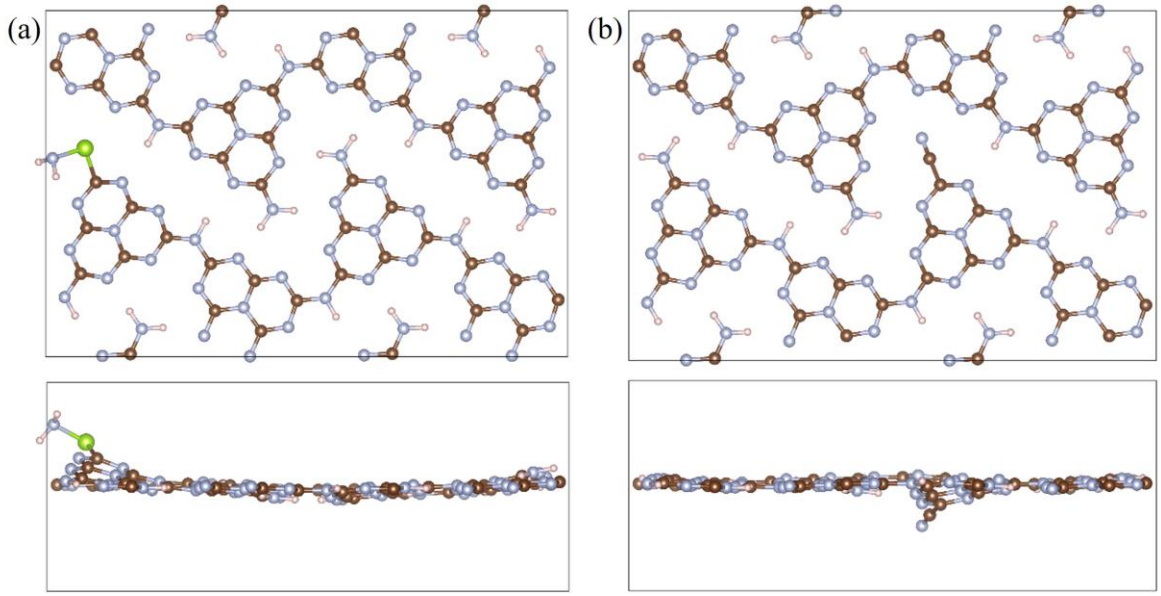

Fig. S2. a). Top and side view of geometric structure of SeCNNS. b). Top and side view of geometric structure of CN. The brown, grey and green color represent carbon, nitrogen and selenium atoms, respectively.

The formation energies (E_{form}) of the SeCNNS were calculated. E_{form} is defined as:

$$E_{form} = E_{SeCNNS} - E_{Se} - E_{CN}$$

E_{SeCN} is the total energy of the SeCNNS (Fig.S2a). E_{Se} is the total energies of the free Se atom, E_{CN} is the total energies of CN (Fig.S2b).

Table S1. The Bader charge of selenium bonding amine group and cyano group in SeCNNS.

Species	Bader charge (|e|)
$SeNH_2$	+0.52
CN	-0.11

Table S2. The physicochemical properties of elements.[6]

Element	Pauling Electronegativity	Atom radius/pm
C	2.25	77
N	3.04	75
Se	2.55	117

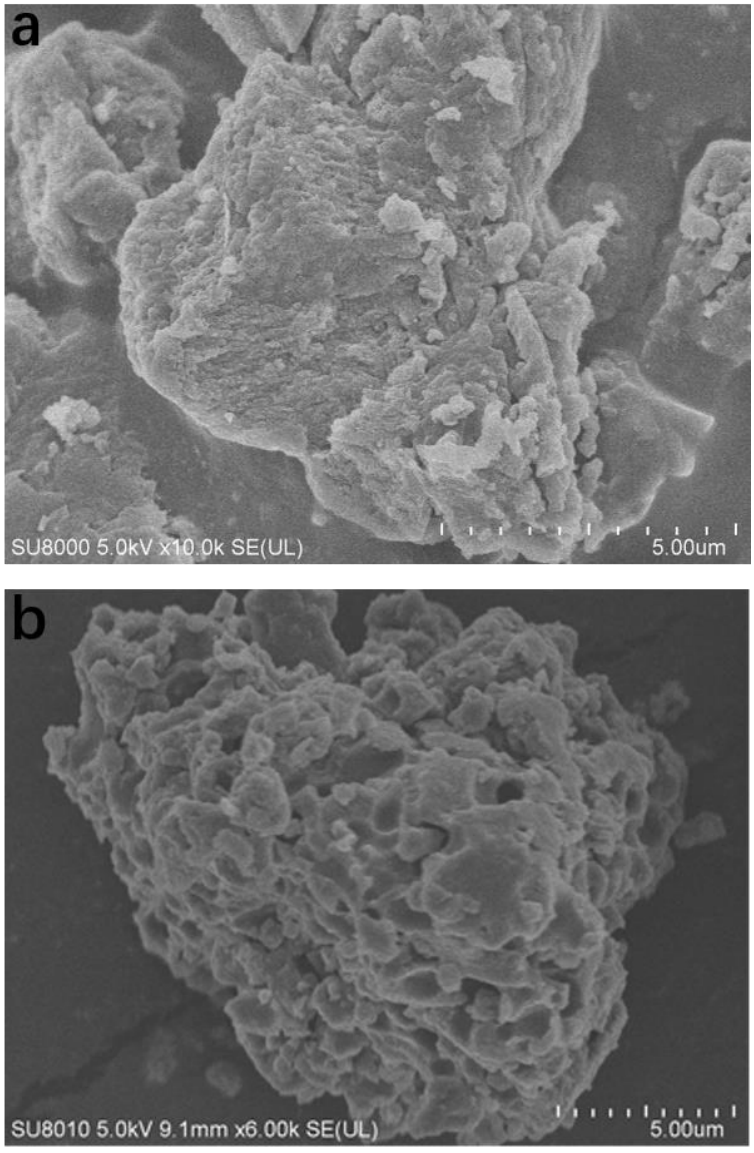

Fig. S3. SEM pictures of CN a) and SeCN b).

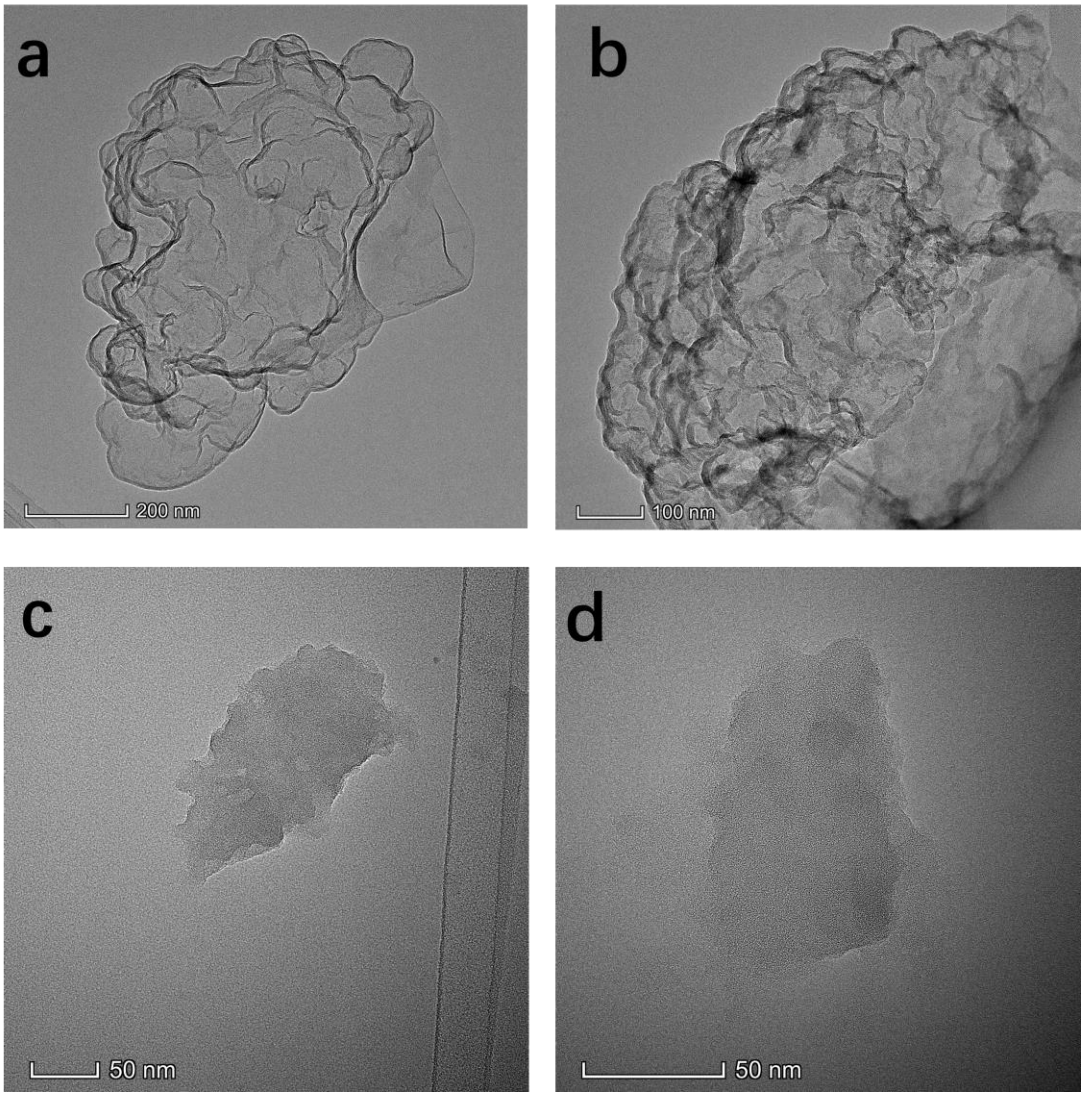

Fig.S4. TEM of SeCN a),b) and SeCNNS c),d).

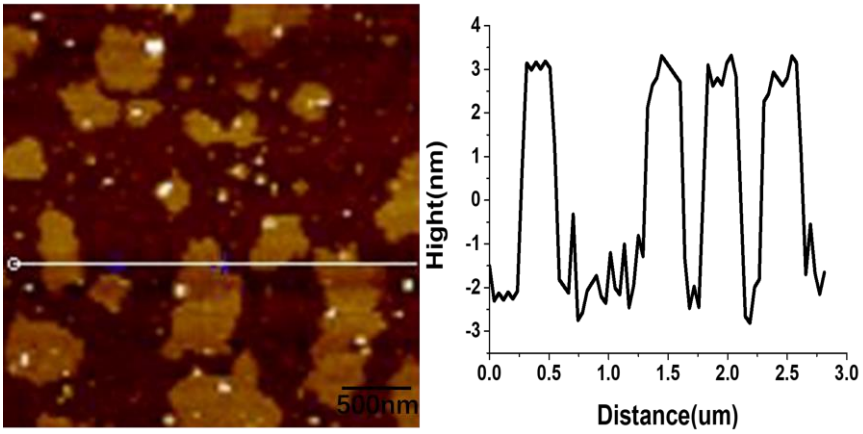

Fig.S5. AFM image of SeCNNS and the corresponding height image of random nanosheets.

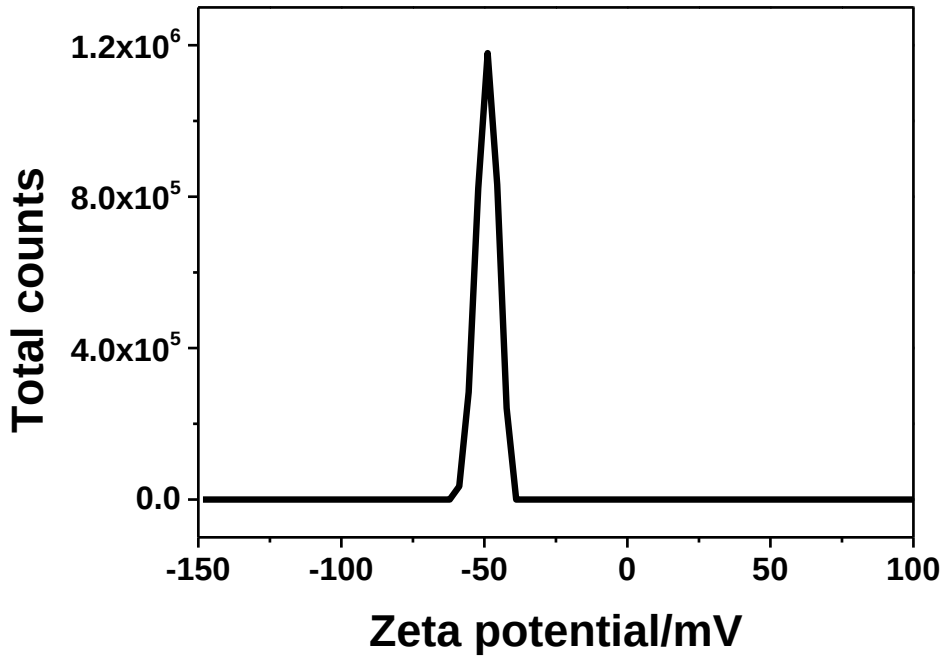

Fig. S6. Zeta potential of SeCNNS solution.

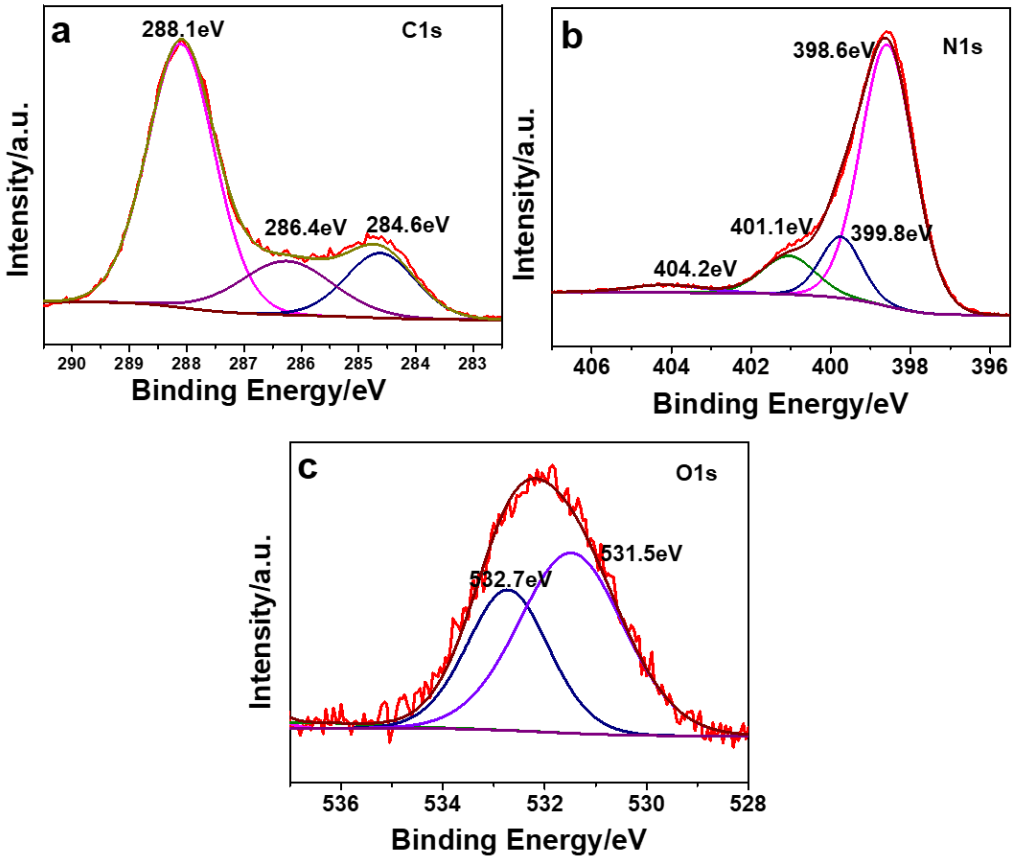

Fig.S7. High resolution XPS spectra of SeCNNS, $C1_S$ a), $N1_S$ b) and $O1_S$ c)

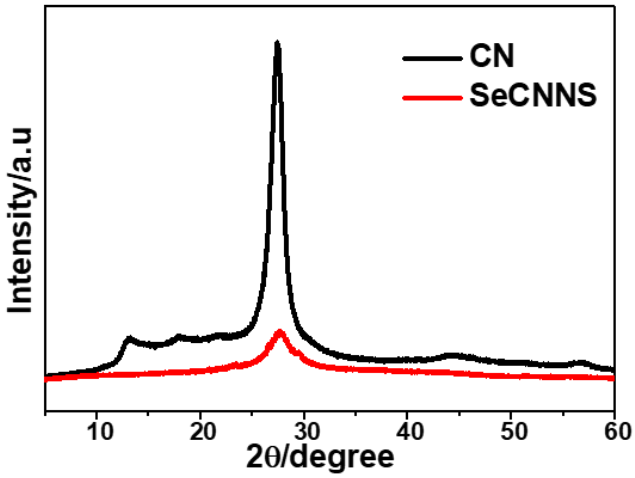

Fig.S8. XRD patterns of bulk CN and SeCNNS.

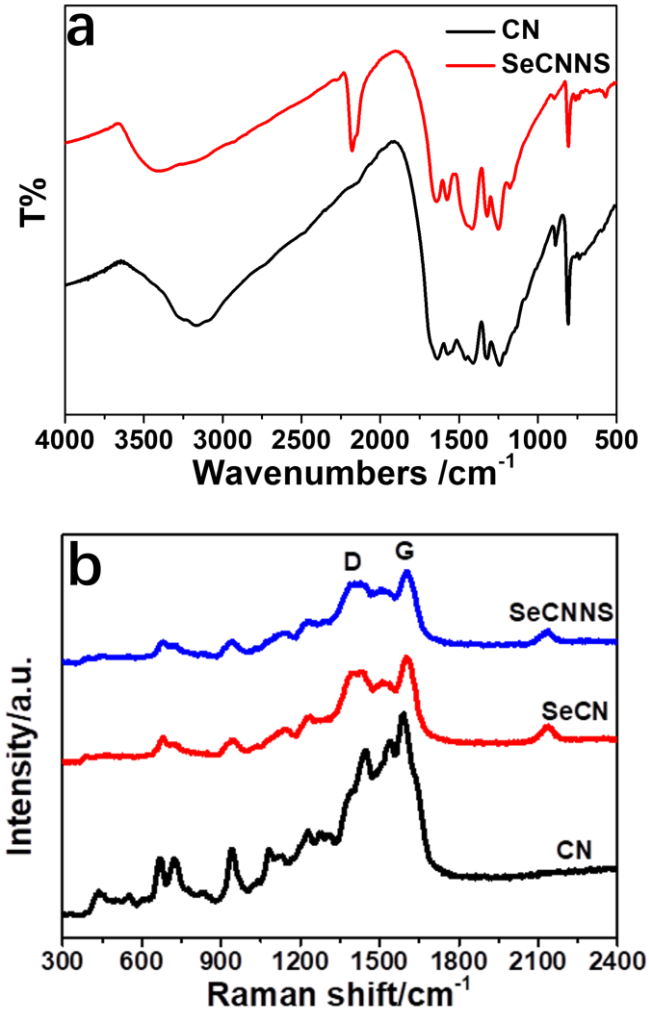

Fig. S9. The FT-IR and UV Raman spectra of SeCNNS, Se-CN and bulk CN.

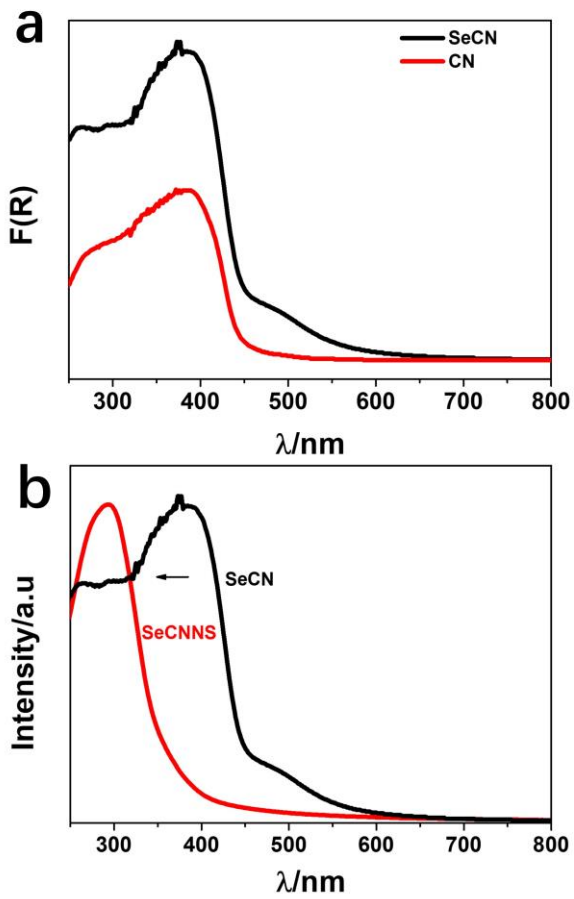

Fig. S10. UV-vis Diffuse Reflection Spectra of CN, SeCN and the UV-vis absorption of SeCNNS solution.

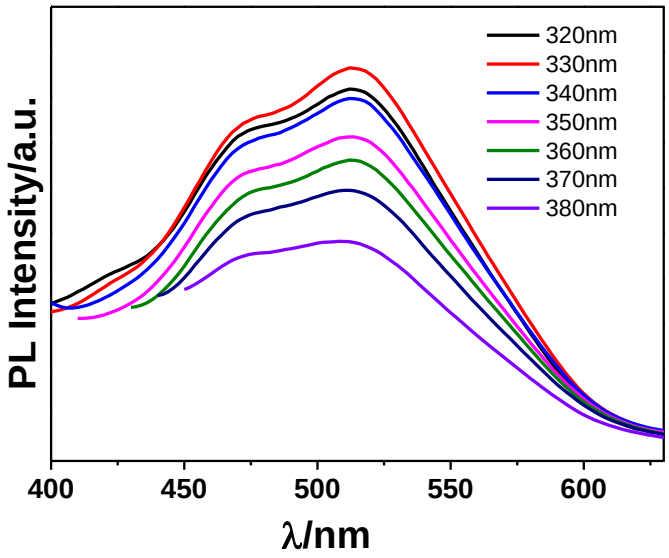

Fig. S11. PL spectra of SeCNNS upon excitation at wavelengths from 320 to 380 nm.

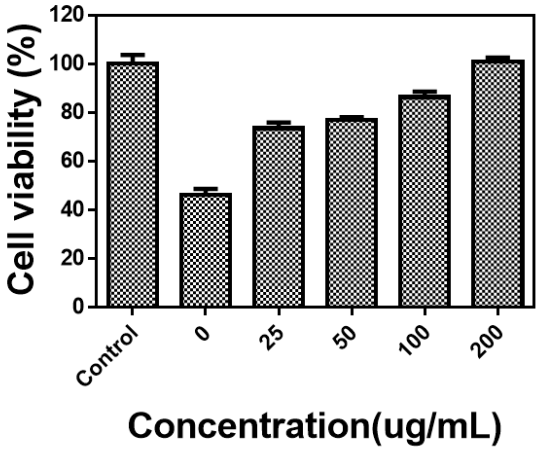

Fig.S12. Cytotoxicity of A549 cells treated with 0.05% H_2O_2 and co-cultured with SeCNNS at different concentrations (0-200 μg/ml).

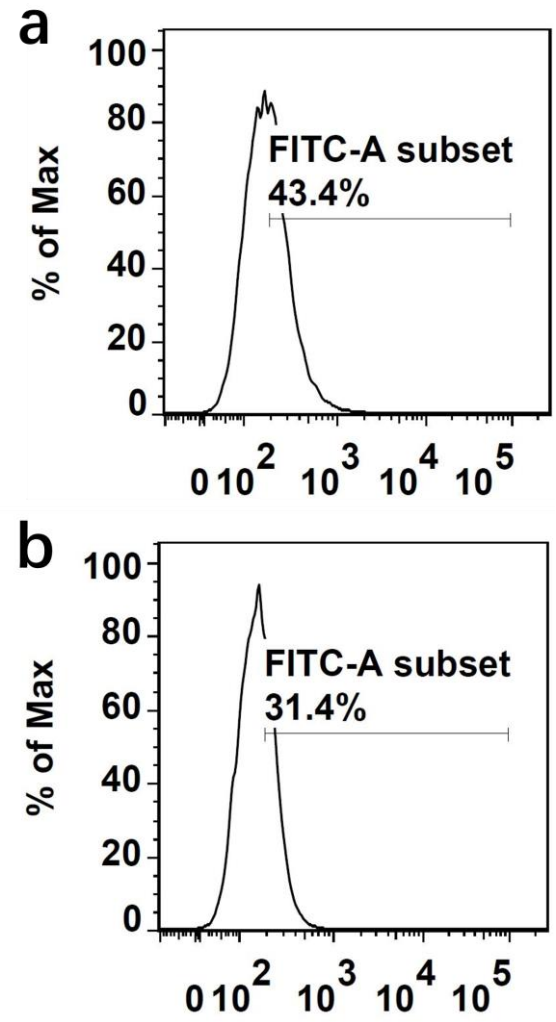

Fig.S13. Flow cytometry for measuring the intracellular ROS concentration of A549 cells treated with 50 μg/ml SeCNNS a), 200 μg/ml b) under 0.015% H_2O_2.

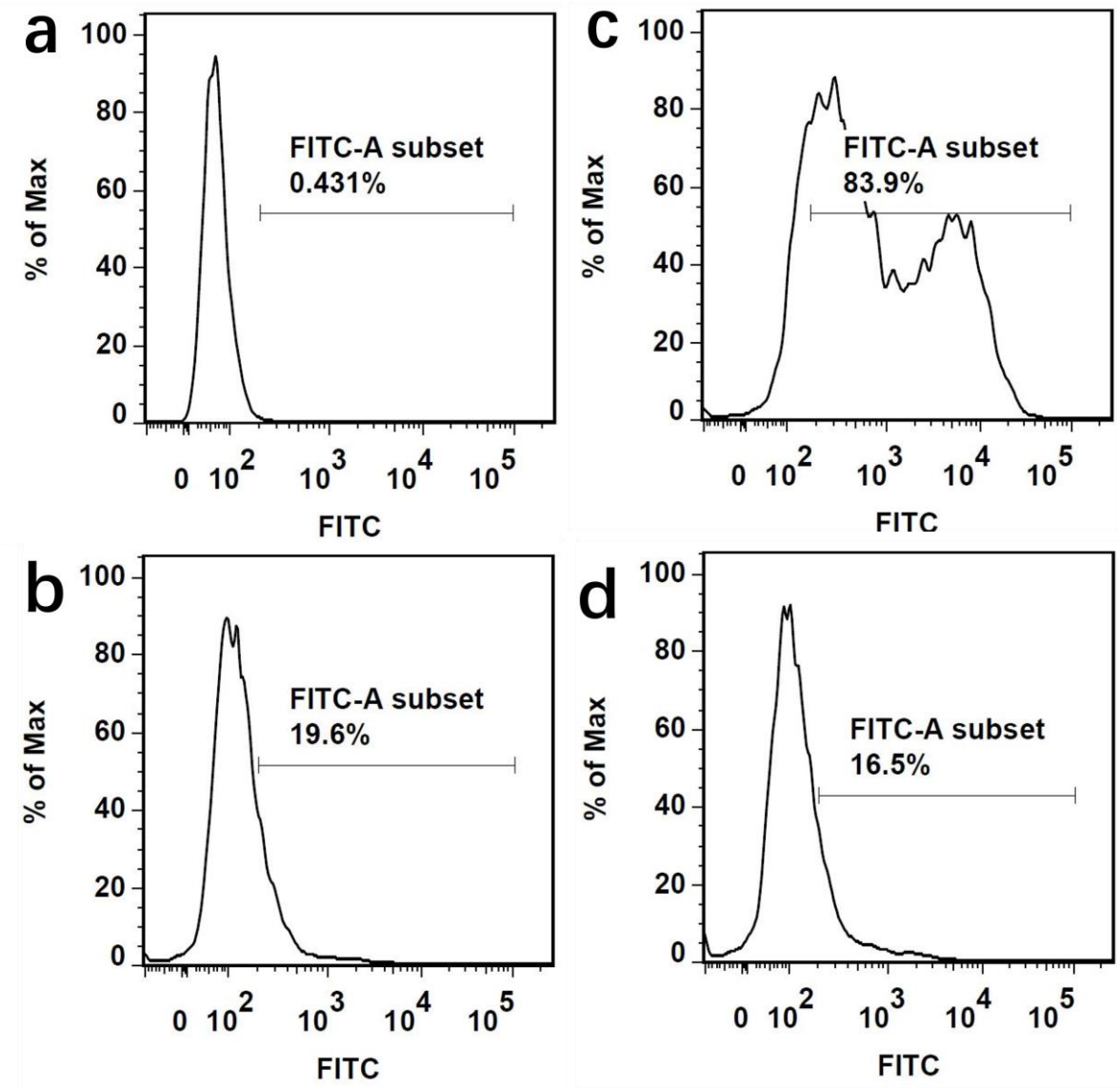

Fig.S14. a) Flow cytometry for measuring the intracellular ROS concentration of A549 cells treated with 50 μ g/ml SeCNNS a), 0.01% H_2O_2 b), 0.01% H_2O_2 and 50μg/ml SeCNNS c), 0.01% H_2O_2 and 100 μg/ml SeCNNS.

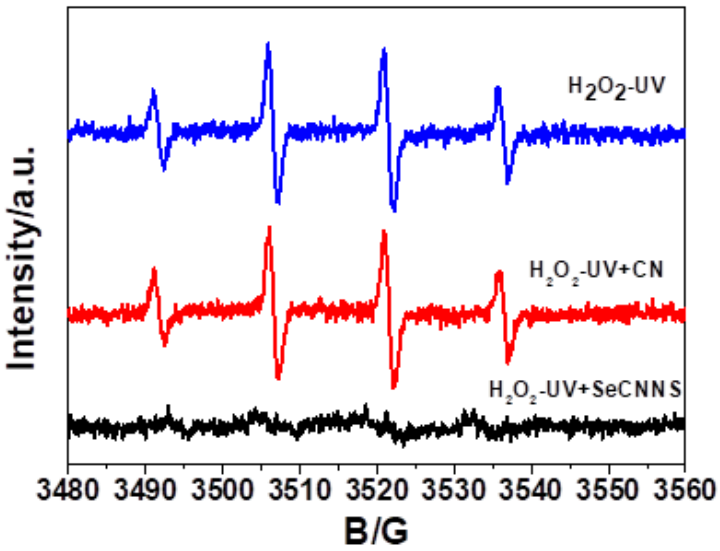

Fig.S15. ESR spectra of DEMPO/•OH with different treatment under UV light irradiation.

References

1. (a)G. Kresse and J. Furthmüller, *Computational Materials Science*, 1996, **6**, 15-50; (b)G. Kresse and J. Furthmüller, *Physical Review B*, 1996, **54**, 11169-11186.
2. J. P. Perdew, K. Burke and M. Ernzerhof, *Physical Review Letters*, 1996, **77**, 3865-3868.
3. J. P. Perdew, M. Ernzerhof and K. Burke, *The Journal of Chemical Physics*, 1996, **105**, 9982-9985.
4. S. Grimme, *Journal of Computational Chemistry*, 2006, **27**, 1787-1799.
5. H. J. Monkhorst and J. D. Pack, *Physical Review B*, 1976, **13**, 5188-5192.
6. H. Ou, C. Tang, Y. Zhang, A. M. Asiri, M.-M. Titirici and X. Wang, *Journal of Catalysis*, 2019, **375**, 104-112.