Supporting Information

Diselenium-containing Ultrathin Polymer Nanocapsules for Highly Efficient Targeting Drug Delivery and Combining Anticancer

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Experimental Section

1.Materials

All the solvents were purchased from Beijing chemical plant. Dichloromethane (DCM), 1, 2-dichloroethane, acetonitrile (MeCN), diethyl ether, Tetrahydrofuran (THF), chloroform ($CHCl_3$) and tetrachloromethane (CCl_4) were used with further purification. Glutathione (reduced) (GSH), Doxorubicin hydrochloride (DOX-HCl), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Hoechst were purchased from Energy Chemical plant. Se powder and sodium borohydride were purchased from Sigma and used as received. Alexa fluor_ 488 annexin V/dead cell apoptosis assay kit was purchased from Invitrogen and used as received. Clear polystyrene tissue-culturetreated 24- and 96-well plates were obtained from Corning Costar. All other reagents and solvents were purchased from the domestic suppliers and used as received.

2. Instruments and methods

^{1}H-NMR spectra, ^{13}C-NMR spectra and ^{77}Se NMR spectra were measured by Bruker 510 spectrometer (500 MHz) using $CDCl_3$ or D_2O as solvent with tetramethylsilane (TMS) as reference. **Mass spectrometry analyses** were performed using liquid chromatograph-mass spectrometer (LC-MS, Agilent1290-micrOTOF-Q Ⅱ) using methanol or acetonitrile (MeCN) or H_2O as solvent. **Fourier transform infrared (FTIR) spectra** were recorded on a Vertex 80V spectrometer. The sample was grinded into powder and dried, then mixture with dried KBr (powder) and pressed into piece. **Dynamic Light Scattering (DLS) experiments** were carried out with Malvern Instrument Zetasizer Nano ZS equipped with a He-Ne laser (633nm, 4mW) and an avalanche photodiode detector. **SEM images** were recorded on scanning electron microscopy, JEOL JSM 6700F. A drop of the aqueous solution was dripped directly onto a silicon wafer and

air-dried. **TEM images** were recorded on a JEM-2100F instrument with an accelerating voltage of 200kV. The sample was prepared by placing a drop of the stock solution on a 300-mesh, carboncoated copper grid and air-dried before measurement. **Fluorescence microscope images** were characterized by Confocal Laser Scanning Microscopy (CLSM) (FV1000 Olympus IX-81). **Apoptosis of cancer cell** was tested by flow cytometry (FACSCalibur).

3. Synthetic procedures

3-1. Synthesis of NaSeSeNa

In a three-necked flask (250 mL), Sodium borohydride (0.567 g, 15 mmol) in 50 mL of water was added. After the system having been completely deoxygenated and in nitrogen atmosphere, selenium powder (1.3 g, 17 mmol) was added. The mixture was stirred for 30 min and then warmed briefly to dissolve selenium. The resulting brownish red aqueous solution of NaSeSeNa was then ready for further use. The chemical reaction equation is shown below:

$2NaBH_4 + 2Se + 6H_2O = NaSeSeNa + 2H_3BO_3 + 7H_2$

3-2. Synthesis of di-tert-butyl (diselanediylbis(ethane-2,1-diyl))dicarbamate

NaSeSeNa solution was added with magnetic stirring to 50 mL of THF solution of tert-butyl (2-bromoethyl)carbamate (2.5 g, 11 mmol) with nitrogen protection. The mixture was stirred for 24 h at 50 ^oC. Then, the solvent was removed, and the crude product was redissolved in CH_2Cl_2. The organic layer was washed twice with water and then dried over anhydrous $MgSO_4$. The product was purified by silica gel column chromatography eluting with 10:1 CH_2Cl_2/ethyl acetate. The evaporation of solvent gave a yellow powder, yield 69%. ^{1}H-NMR (500 MHz, chloroform-d, 25℃, TMS) (Figure S1) δ: 3.57 (4H, -Se$CH_2$$CH_2$NH), 3.05 (4H, CH_2CH_2NH), 1.51(2H, CH_2CH_2NH),

1.41 (18H, OC($\boldsymbol{CH_3}$)$_3$). ESI-MS (Figure S2): calculated for C4H12N2Se2, m/z 448.6, found m/z 448.4 $[M]^+$, m/z 471.6 $[M+Na]^+$.

3-3 Synthesis of 2,2'-diselanediylbis(ethan-1-amine) (DiSe-Diamine)

Di-tert-butyl (diselanediylbis(ethane-2,1-diyl))dicarbamate is added into solution of 10% trifluoroacetic acid (TFA)/$CHCl_3$, the reaction was stirred for 4 h at 25 °C. Then, the solvent was removed, and the crude product was purified by recrystallization. ^{1}H-NMR (500 MHz, chloroform-d, 25℃, TMS) (Figure S3) δ: 3.37 (4H, -SeCH$_2$$\boldsymbol{CH_2}NH_2$), 3.13 (4H, -Se$\boldsymbol{CH_2}CH_2NH_2$). ESI-MS (Figure S4): calculated for C4H12N2Se2, m/z 247.9, found m/z 248.9, $[M+H]^+$.

3-4. Synthesis of DOX-DiSe-CAP

DiSe-Diamine (60 mg) and BDMP5 (50 mg) and DOX-HCl (40 mg) were dissolved into 50 mL MeCN. Then the reaction was added into 2 mL Triethylamine and stirred at 70 °C for 5 h in nitrogen atmosphere. After cooling to room temperature, the solution was dialyzed against water for 24 h to remove the MeCN, residual DOX and other unreacted agents. After that the solution was filtered to give the final DOX-DiSe-CAP. The drug-loading efficiency (LE) and drug-loading content (DLC) are calculated as follow formula:

$$LE\ (\%) = \frac{amount\ of\ DOX\ loaded}{amount\ of\ DOX\ feeding} \times 100\%$$

$$DLC\ (\%) = \frac{weight\ of\ drugs\ in\ the\ nanocapsules}{weight\ of\ the\ whole\ nanocapsules} \times 100\%$$

3-5. Synthesis of RGDlg- DOX-DiSe-CAP

DOX-DiSe-CAP (20 mg) and RGD-ligand (10 mg) and were dispersed into 10 mL $CHCl_3$. Then the reaction was stirred at room temperature for 36 h. Then the solvent was removed by

rotary evaporation. After that, the obtained solid was dissolved in water and dialyzed against water to remove the residual RGD-ligand.

3-6. MTT assay

The cells were seeded into 96-well plates at 8×10^3 cells per well in 200μL of culture medium. After incubation, the medium was removed and replaced with another 200 μL of culture medium containing serial drugs. Cells without the treatment were used as control. The cells were grown further for different incubation times. Then, 20 μL of 5 mg/mL MTT assay stock solution in PBS was added to each well. After the cells were incubated for 4 h, the medium containing unreacted MTT was carefully removed. Then, the obtained blue formazan crystals were dissolved in 200 μL $well^{-1}$ DMSO, and the absorbance was measured in a BioTek Synergy H4 hybrid reader at a wavelength of 490 nm. The blank was subtracted to the measured optical density (OD) values, and the cell viability was expressed as % of the values obtained for the untreated control cells.

3-7. Cellular uptake

For cellular uptake, MCF-7 cells were seeded in 24-well plate with the cell density of 2.5×10^5 cells per well. The cells were then incubated at 37 ^oC for 24 h in a humidified atmosphere. 200 μL of DOX, DOX-DiSe-CAP and RGDlg- DOX-DiSe-CAP were added into the plate respectively. The cells without treatment were used as control. After another 4 h of incubation at 37 ^oC, the cells were washed with PBS (0.01M, pH=7.4) three times. Hoechst was used to stain the nucleus of the cells. The cells were then observed by confocal laser scanning microscopy (CLSM). The experiment was repeated three times.

3-8. Apoptosis assay

MCF-7 cells were seeded in 6-well plates at 5×10^5 cells per well in 2 mL of complete DMEM and cultured for 24 h, followed by removing culture medium and adding 2 mL DiSe-CAP or DiSe-Diamine solution of DMEM at various concentrations from 0.0001 to 0.2 mg/mL. MCF-7 cells without treatment were used as control. After 24 h incubation, cells were rinsed by PBS twice and treated with trypsin. Then, 2 mL of cold PBS was added to each culture well, and the solutions were centrifugated at 1000 rpm for 5 min at 4 ^oC. After the removal of supernatants, the cells were resuspended in annexin-binding buffer at about 1×10^6 cells mL^{-1}. Then, 5 μL of Alexa Fluor^R 488 annexin V and 1μL of 100 mg/mL PI working solutions were added to each 100 μL of the cell suspension. After 15 min incubation at room temperature, 400 μL of annexin-binding buffer was added. The samples were kept on ice and analyzed by flow cytometry. Data for 5000 gated events were collected, and the analysis of live and dead cells was performed by means of a BD FACSCalibur flow cytometer and CELLQuest software.

3-9. Preparing of DiSe-Cap

DiSe-Diamine (**6**0 mg) and BDMP5 (50 mg) and DOX-HCl (40 mg) were dissolved into **5**0 mL MeCN. Then the reaction was added into **2** mL Triethylamine and stirred at 70 ^oC for 5 h in nitrogen atmosphere. After cooling to room temperature, the solution was dialyzed against water for 24 h to remove the MeCN and other unreacted agents.

3-10. Preparing of Cap-1

The Cap-1is synthesized according to our previous work.[1] Hexanediamine (15.7 mg) and BDMP5 (30 mg) were dissolved into 30 mL MeCN. Then the reaction was stirred at 50 ^oC for 5 h in nitrogen atmosphere. After cooling to room temperature, the solution was dialyzed against water for 24 h to remove the MeCN and other unreacted agents.

3-11. Preparing of disulfide-Cap

The pillar[5]arene-based single molecular layer polymer nanocapsules with disulfide bridgehe is synthesized according to our previous work[2]. Cystamine dihydrochloride (21mg) and BDMP5 (30 mg) were dissolved into 30 mL MeCN. Then the reaction wasadded into 2 mL Triethylamine and stirred at 70 ^{o}C for 5 h in nitrogen atmosphere. After cooling to room temperature, the solution was dialyzed against water for 24 h to remove the MeCN and other unreacted agents.

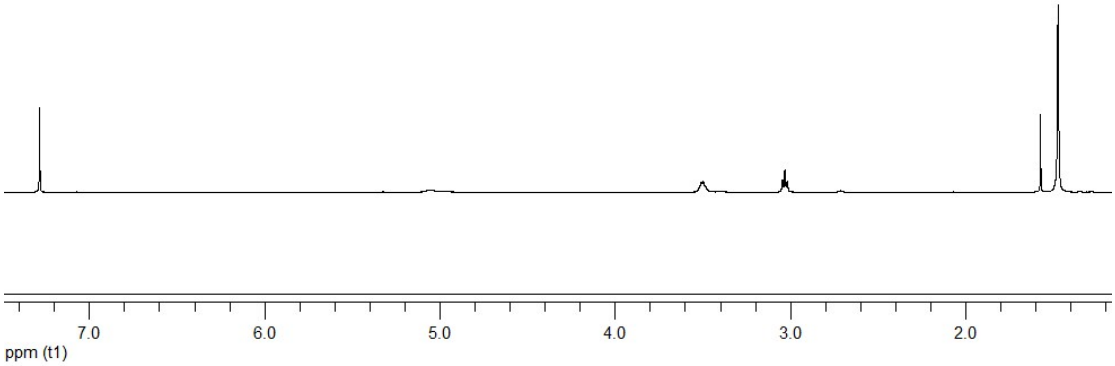

Figure S1. The ^{1}H-NMR (500MHz, chloroform-d, 25℃, TMS) spectrum of di-tert-butyl (diselanediylbis(ethane-2,1-diyl))dicarbamate .

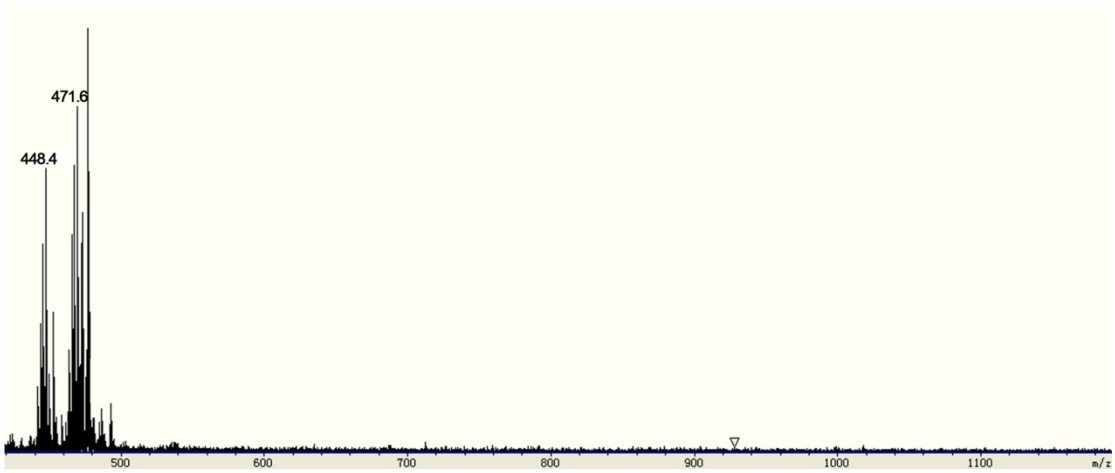

Figure S2. ESI-tof of di-tert-butyl (diselanediylbis(ethane-2,1-diyl))dicarbamate. Calculated for C4H12N2Se2, m/z 448.6, found m/z 448.4 $[M]^{+}$, m/z 471.6 $[M+Na]^{+}$.

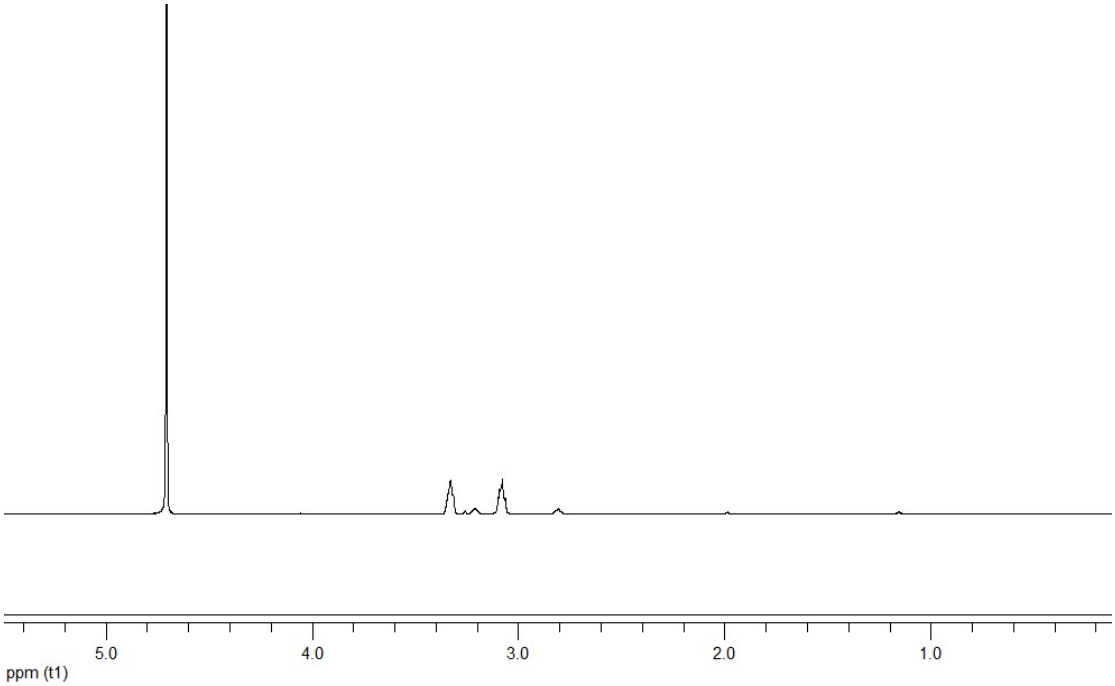

Figure S3. The ^{1}H-NMR (500MHz, D_2O, 25℃, TMS) spectrum of DiSe-Diamine.

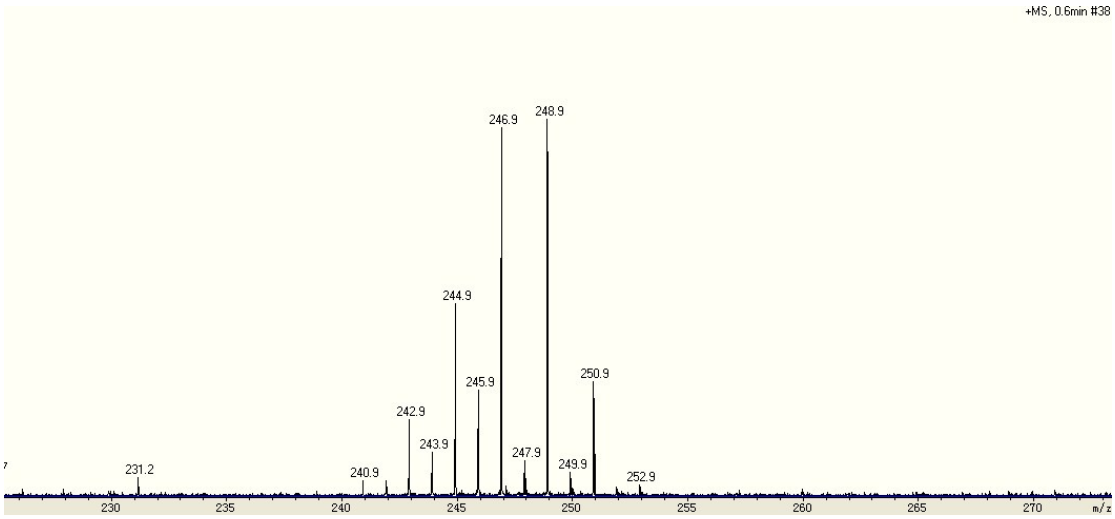

Figure S4. ESI-tof of the DiSe-Diamine. Assignment of the main peak: m/z 248.9，$[M+H]^+$.

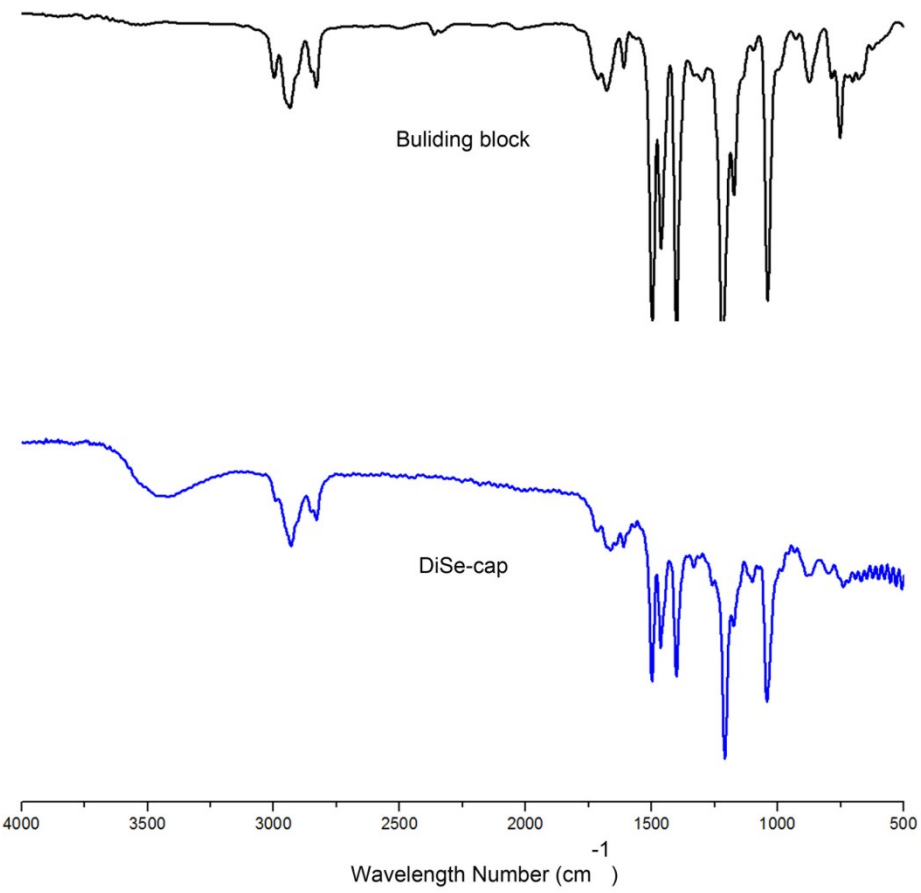

Figure S5. Fuorier transform IR spectrum of BDMP5 (black line) and purified DiSe-Cap(blue line).

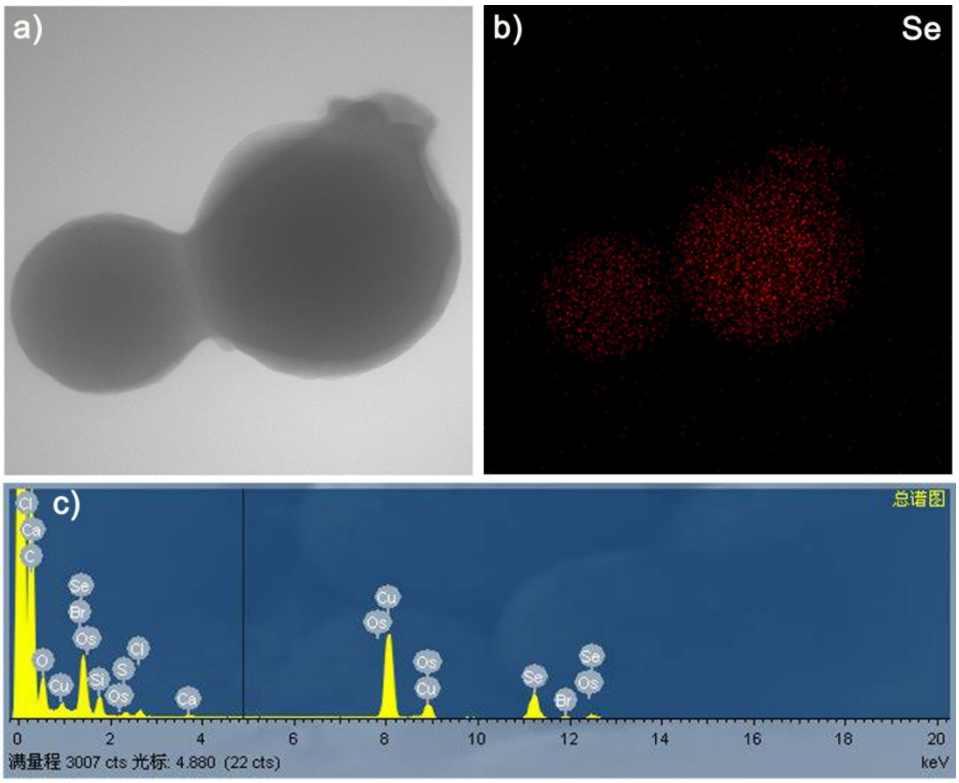

Figure S6. a) Dark-field TEM images of DiSe-cap; b) matching TEM elemental mappings for Selenium element; c) EDX spectrum analysis of the DiSe-cap.

Reference

(1) S. Fu, G. An, H. Sun, Q. Luo, C. Hou, J. Xu, Z. Dong and J. Q. Liu, *Chem. Commun.,* 2017, **53**, 9024.

(2) S. Fu, Y. Zhang, S. Guan, Q. Huang, R. Wang, R. Tian, M. Zang, S. Qiao, X. Zhang, S. Liu, X. Fan, X. Li, Q. Luo, C. Hou, J. Xu, Z. Dong and J. Q, Liu, *ACS Appl. Mater. Interfaces,* 2018, **10**, 14281.