Supporting information

for

ROS-responsive organosilica nanocarrier for targeting-delivery of metformin against cancer with synergistic effect of hypoglycemia

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Contents

Experimental Section

Synthesis of ROS-cleavable thioketal (TK)

The ROS-cleavable thioketal (TK) was synthesized according to a previous study.[1] Briefly, anhydrous acetone (3.40 g, 58.60 mmol) and anhydrous 3-mercaptopropionic acid (3.00 g, 28.30 mmol) were dissolved under a dry HCl atmosphere. After stirring for 6 h at room temperature, the as-prepared product was crystallized in an ice saturated sodium chloride bath, followed by washes with hexane (3 × 100 mL) and cold water (3 × 100 mL). After freeze-drying, 3,3'-(propane-2,2-diylbis(sulfanediyl))dipropionic acid (TK) was obtained as a white solid.

Synthesis of TK-NHS

TK (3.00 g, 11.95 mmol), NHS (3.30 g, 28.69 mmol), and EDCI (6.87 g, 35.86 mmol) were successively added into DCM (100 mL). After overnight stirring at room temperature, the reaction mixture was washed twice successively with 1.0 M HCl, saturated $NaHCO_3$, and saturated NaCl. The organic phase was then collected and dried over anhydrous $MgSO_4$, followed by filtering. Finally, the solvent was removed using a rotary evaporator, resulting in TK-NHS as a white solid.

Synthesis of ROS-cleavable bridge-organoakloxysilane

APTES (2.97 g, 13.44 mmol) was added dropwise to a solution of TK-NHS (3.00 g, 6.72 mmol) in anhydrous DCM (100 mL) under argon atmosphere. After overnight stirring at room temperature, the solvent was evaporated under reduced pressure and ROS-cleavable bridge-organoakloxysilane was obtained as a pale yellow oil.

Synthesis of RGD-PEG_{2000}-silane

NHS-PEG_{2000}-silane (100 mg, 0.04 mmol) was dissolved in a solution of anhydrous DCM (5 mL). The solution of RGD (24.17 mg, 0.04 mmol) in anhydrous DMF (3 mL) was then added dropwise to the reaction system under an argon atmosphere. After overnight stirring at room temperature, the as-prepared product was precipitated using cool diethyl ether. The pale yellow solid was obtained by centrifugation, washed twice with cool diethyl ether, and vacuum dried overnight.

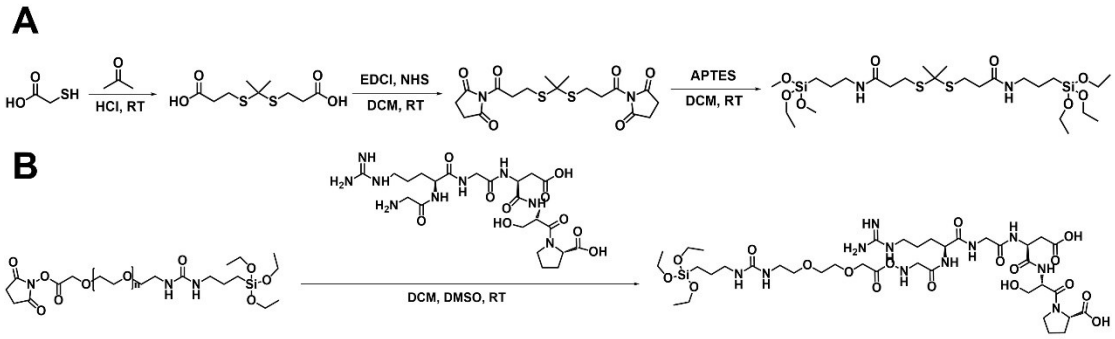
Ellman's procedure

The T-BS-NPs treated with or without H_2O_2 were analyzed by Ellman's reagent.[2] Briefly, 50 mg of materials was added to a solution of 0.1 g Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid, DNTB) and 500 μl of N,N-diisopropylethylamine (DIPEA) in approximately 50 ml of methanol. The mixture was mechanically shaken for 24 h. The absorbance of the solutions was measured at 412 nm.

Cell culture

HeLa cell line was purchased from Cell Bank of the Chinese Academy of Science (Shanghai, China). Cells were cultured in DMEM media (10 mM glucose) with 10% FBS unless otherwise indicated. For starvation experiments, HeLa cells were grown in glucose-free DMEM supplemented with 10% FBS and 2.5 mM L-glutamine unless otherwise indicated. All cultures were maintained at 37°C under a humidified atmosphere with 5% CO_2.

Supporting Figures and Tables



Scheme S1. Synthetic route of (A) bridged organoakloxysilane and (B) RGD-PEG_{2000}-silane.

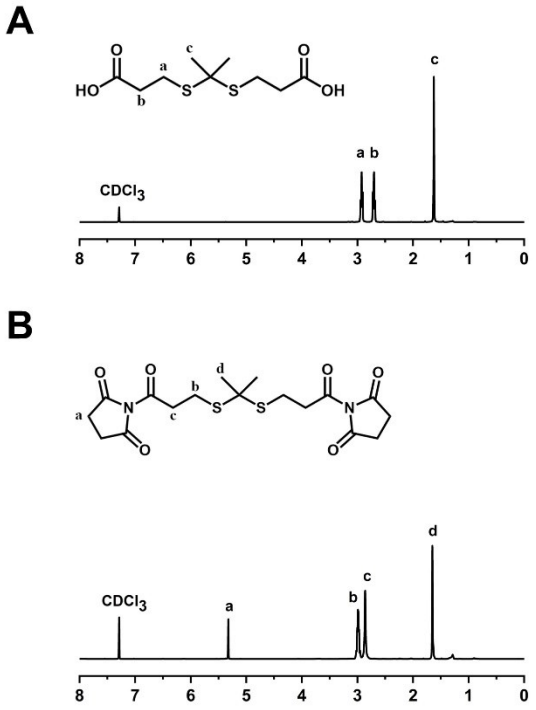


Fig. S1. ^{1}H-NMR spectrum of (A) TK, (B)TK-NHS.

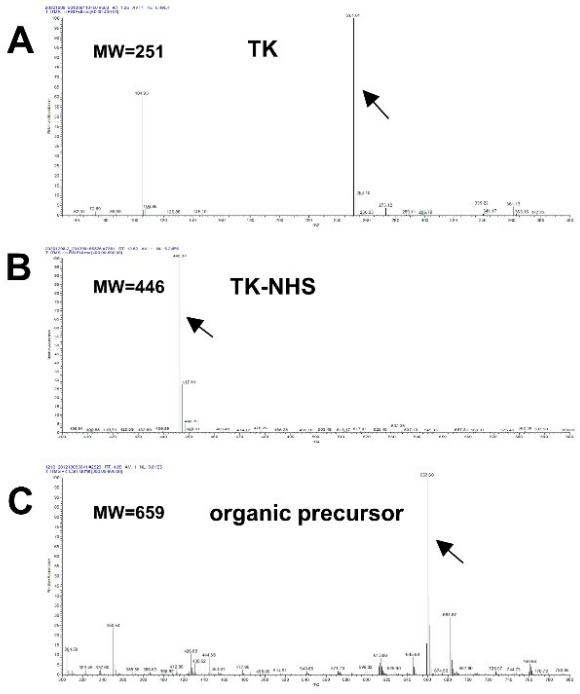


Fig. S2. MS spectrum of (A) TK, (B) TK-NHS, and (C) organic precursor.

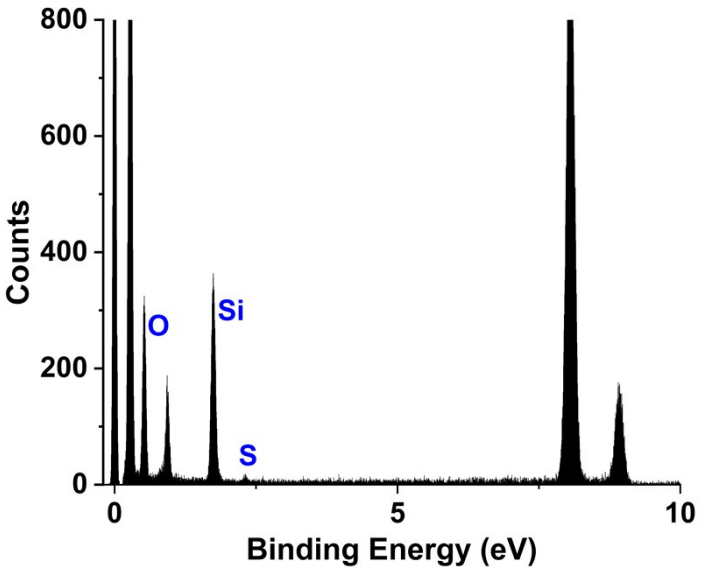


Fig. S3. Energy-dispersive X-ray spectroscopy (EDS) spectra of T-BS-NPs.

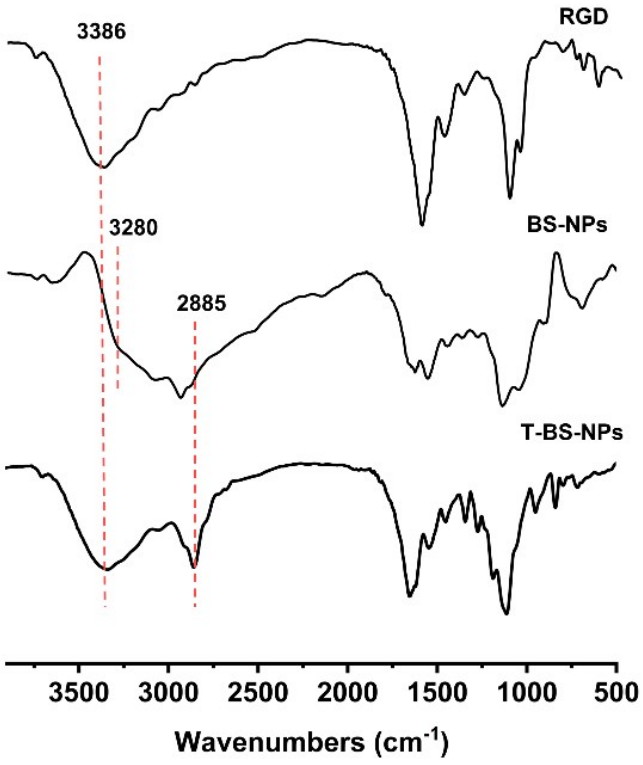


Fig. S4. FTIR spectrum of RGD, BS-NPs, and T-BS-NPs.

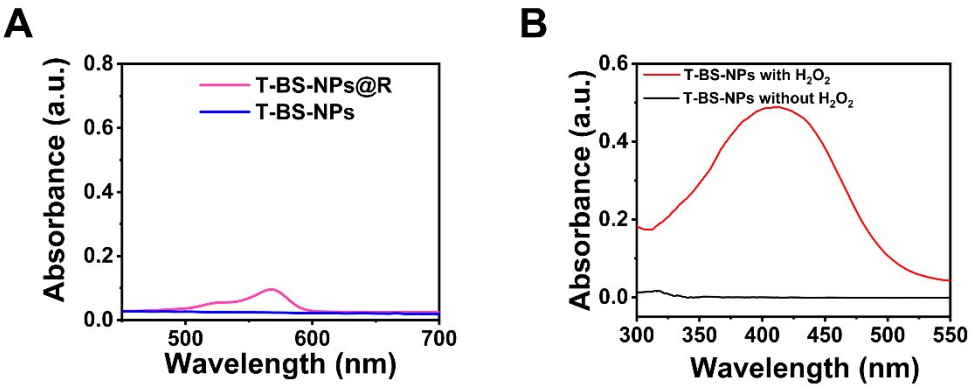


Fig. S5. UV-visible spectrum of T-BS-NPs and T-BS-NPs@R in water and (B) thiol group in the T-BS-NPs treated with H_2O_2.

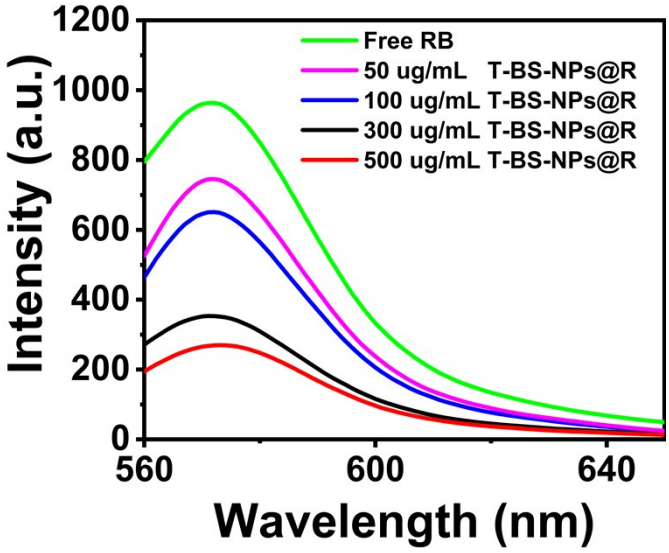


Fig. S6. Fluorescence spectra of T-BS-NPs@R with different concentration.

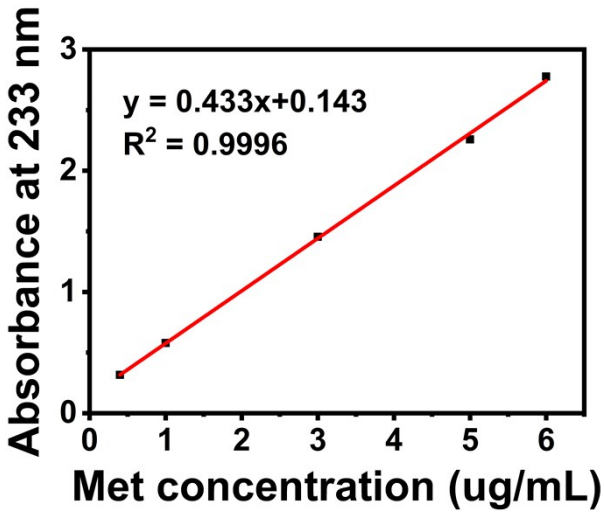


Fig. S7. The concentration (μg/mL) of metformin as a function of the absorbance at a wavelength of 233 nm.

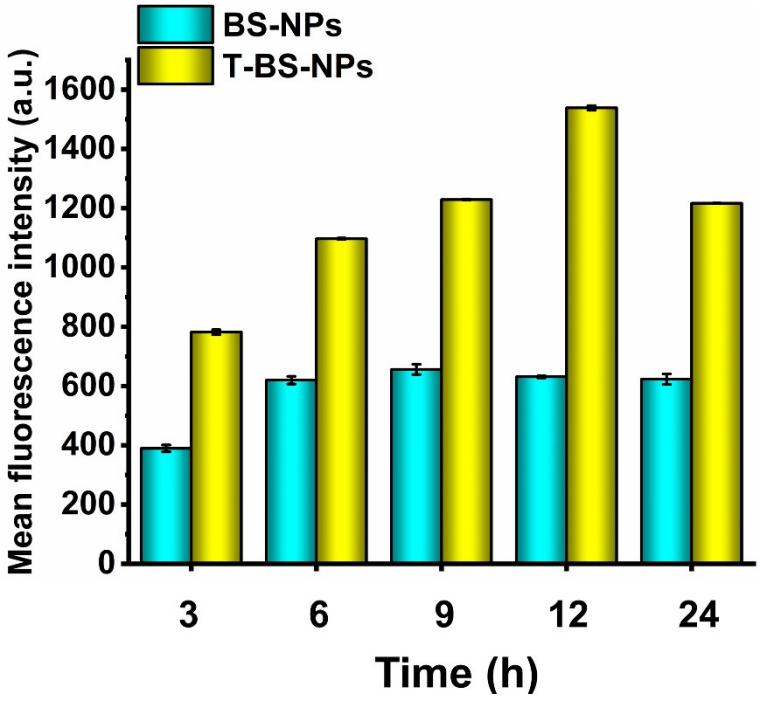


Fig. S8. Mean fluorescence intensities in the tumor areas of HeLa tumor-bearing mice at different time after injection of BS-NPs@R and T-BS-NPs@R.

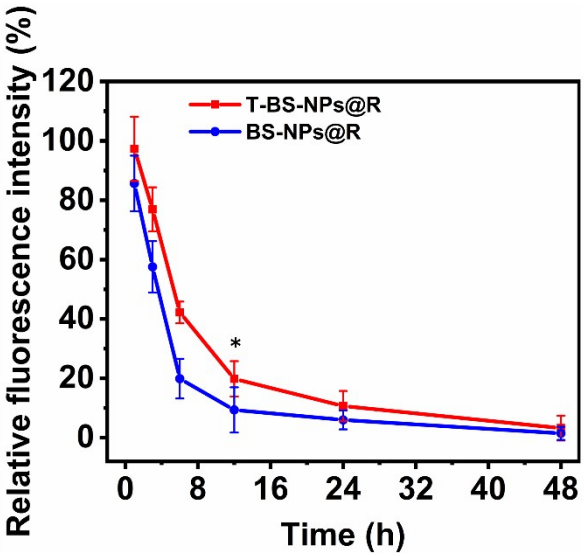


Fig. S9. Relative fluorescence intensity of RB in blood at various time points after i.v. injection of BS-NPs@R and T-BS-NPs@R nanoparticles. (n = 3; means ± SD; *P < 0.05, **P < 0.01, ***P < 0.001). Statistical analyses were performed using analysis of variance (ANOVA) with Tukey's test.

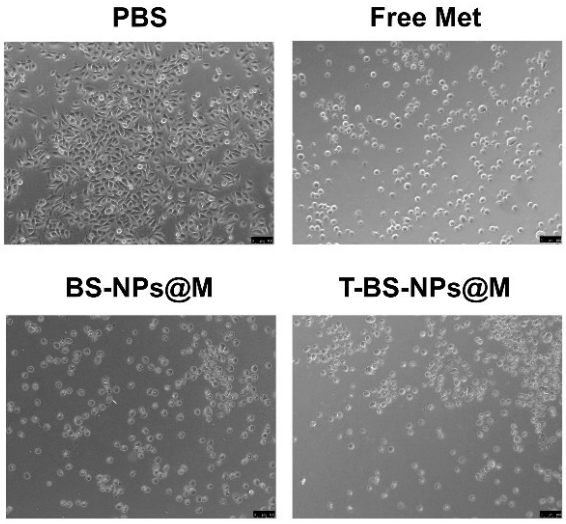


Fig. S10. Images of starved HeLa cells cultured with PBS, free metformin, BS-NPs@M, and T-BS-NPs@M for 24 h. Media were replenished every 6 h. Scale bars = 100 μm.

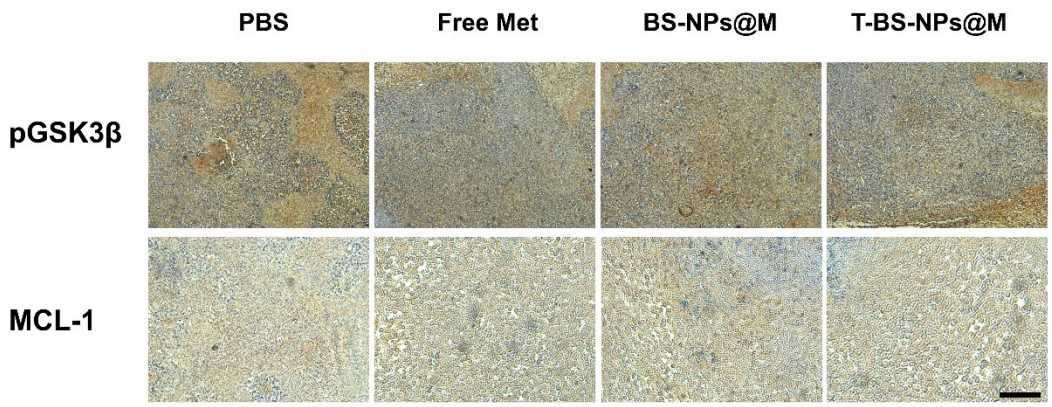


Fig. S11. Immunohistochemistry analyses of pGSK3β and MCL-1 proteins in tumor tissue sections from CTRL group. Scale bar = 250 μm.

Table S1. Resource of antibody.

Antibodies	Source	Identifier
Anti-GSK3 beta antibody [Y174]	Abcam	Cas. #: ab32391
Anti-GSK3 beta (phospho S9) antibody	Abcam	Cas. #: ab107166
Anti-MCL1 antibody [Y37]	Abcam	Cas. #: ab32087
Anti-CIP2A antibody	Abcam	Cas. #: ab99518
Anti-PPP2R5D antibody [EPR15617]	Abcam	Cas. #: ab188323
Anti-beta Actin antibody [AC-15]	Abcam	Cas. #: ab6276

References

1. J. J. Hu, Q. Lei, M. Y. Peng, D. W. Zheng, Y. X. Chen and X. Z. J. B. Zhang, *Biomaterials*, 2017, **128**, 136-146.

2. D Esquivel, Ouwehand, J. , Meledina, M. , Turner, S. , Van Tendeloo, G. , and Romero-Salguero, F. J. , *Journal of Hazardous Materials*, **339**, 368.