Electronic Supplementary Information (ESI)

A New Strategy to Prepare Glutathione Responsive Silica Nanoparticles

Zhigang Xu[a], Kelin Zhang[a], Xiaoyan Liu[a], Haixia Zhang[*a]

[a] State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, China

[*] *E-mail address:* zhanghx@lzu.edu.cn (Haixia Zhang)

Experimental Section

Reagents and Materials

Cystamine dihydrochloride (CADHC, 97 %) and 3-(Triethoxysilyl)propyl isocyanate (TOPI, 95 %) were from alfa aesar, and used without further purifications. Tetrahydrofuran (THF, 99.5 %) and triethylamine (TEA, 99%) purchased from Tianjin Chemical Reagent (China) were used after being stirred overnight over CaH_2 and distilled under reduced pressure. Ethanol (C_2H_5OH, 99.7%) and ammonium hydroxide ($NH_3 \cdot H_2O$, 28%) were supplied by Tianjin Guangfu Chemical Reagent Co. Ltd. (China). Tetraethyl orthosilicate (TEOS, 99.99 %) and glutathione (GSH, 98%) was purchased from Aladdin Reagent Co. Ltd. (China). All these reagents were of analytical grades. Deionized (D.I.) water was prepared from Millipore (Bedford, MA, USA).

Synthesis of disulfide bond bridged silane (BTOCD)

In a flask with 80 mL of THF, cystamine dihydrochloride (1.0 g, 4.5 mmol) and triethylamine (1.25 mL) were added with stirring at room temperature. After 1.0 h, 2 equivalents of 3-(Triethoxysilyl)propyl isocyanate (1.2 mL, 9.0 mmol) were added then stirred for 24 h. The solution was then filtered to remove the salt, and the crude

product was recovered by evaporation of THF. The resulting BTOCD was isolated by precipitation in cold diethyl ether, filtration, and drying in vacuo. Yield: 2.9 g, 85 %. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.66 (s, 4H, $SiCH_2$), 1.22 (t, 18H, CH_3), 1.69 (m, 4H, CH_2), 2.79 (m, 4H, SCH_2), 3.15 (m, 4H, $CONHCH_2$), 3.54 (m, 4H, CH_2CONH), 3.54 (m, 4H, CH_2CONH), 3.83 (q, 12H, $SiOCH_2$), 5.31 (s, 2H, CONHC), 5.32 (s, 2H, $CH_2NHCONH$). ^{13}C NMR (DMSO-d_6, 600 MHz): 157.9, 57.7, 45.7, 42.0, 36.4, 23.5, 18.2, 7.2.

General method of preparing 50 nm-sized silica nanoparticles

Typically, 60 mg of BTOCD and 215 mg of tetraethyl orthosilicate (TEOS) were dissolved in a dry ethanol and 0.25 ml of ammonia and 0.25 ml of water were added with stirring. After 12 hours of stirring, silica nanoparticles were isolated by centrifugation at the speed of 15000 r/min, and the supernatant was removed. The isolated products were redispersed in ethanol. The washing and dispersion was repeated 3 times. Finally, the nanoparticle solution was centrifuged at the speed of 4000 r/min to remove any aggregated particles. The purified silica nanoparticles were homogeneously dispersed in ethanol. The size and shape of the nanoparticles were characterized by TEM. Synthetic procedures were similar for 200 nm size silica nanoparticles, except the concentration of TEOS, water, ammonia and string speed. Table S1 summarizes the synthesis conditions for silica nanoparticles.

Characterization

The ^{1}H NMR and ^{13}C NMR spectra of the sample were recorded on a Bruker AVANCE 600MHz spectrometer (Rheinstetten, Germany) using tetramethylsilane as an internal standard at 25 ℃. The Fourier transform infrared (FTIR) spectra were acquired on a Nicolet 20 NEXUS 670 FT-IR spectrophotometer (Ramsey, MA, USA) using KBr pellets. The morphology of the samples was recorded by a JEM-1230EX transmission electron microscopy (TEM) (Japan), and samples for TEM measurements were made by casting one drop of the sample's ethanol solution on carbon-coated copper grids. Absorption spectra were carried out using a Puxi UV-1810 visible spectrophotometer (Beijing, China).

In Vitro degradation Study

The biodegradation study was achieved as follows: a known concentration of silica NPs-50 or Silica NPs-200 was added into 5 mL of PBS (pH 7.4) solution with a GSH concentration of 8 mM and placed for 1 day, 3 days and 5 days at room temperature, respectively. At a predetermined time, a drop of the above solution was taken for TEM measurement.

Hemolysis Assay in Vitro

The *in vitro* hemolysis of silica NPs was measured to evaluate the blood compatibility. Fresh human blood stabilized with ethylenediamine tetraacetic acid (EDTA), kindly provided by Gansu Blood Center (China). First, 2 mL of blood sample was added to 4 mL of sterile isotonic PBS, and then the serum was removed from human red blood cells (HRBCs) by centrifugation at 3000 r/min for 10 min, the HRBCs were further washed five times with 4 mL sterile isotonic PBS solution and this procedure was repeated more than five times to ensure the removal of any released hemoglobin[1]. Following the last wash, the purified blood was diluted to 20 mL of sterile isotonic PBS. Herein, HRBCs incubation with D.I. water and PBS were used as the positive and negative controls, respectively. Then 0.2 mL of the diluted HRBCs suspension was mixed with: a) 0.8 mL of D.I. water as a positive control; b) 0.8 mL PBS as a negative control; c) 0.8 mL silica NPs at concentrations of 6.25, 31.25, 156.25, 312.5 and 625 μg/mL. All the sample tubes were kept in static condition at room temperature for 3 h. Finally, the mixtures were centrifuged at 3000 r/min for 10 min, and the absorbance values of the supernatants at 576 nm were determined by UV-visible absorption spectrum. The percent hemolysis of the HRBCs was calculated according to the equation below[2]:

$$\text{Hemolysis } \% = \frac{(\text{Sample absorbance- Control absorbance})}{(\text{Positive control absorbance- Control absorbance})} \times 100\%$$

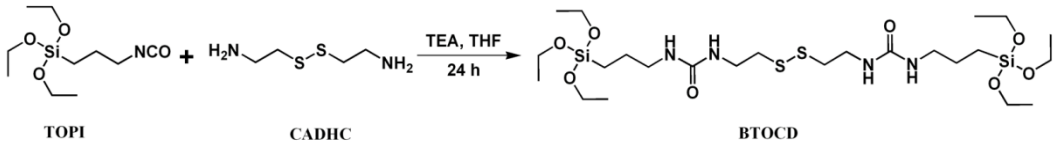

Figure S1 Synthesis protocol of disulfide bond bridged silane (BTOCD)

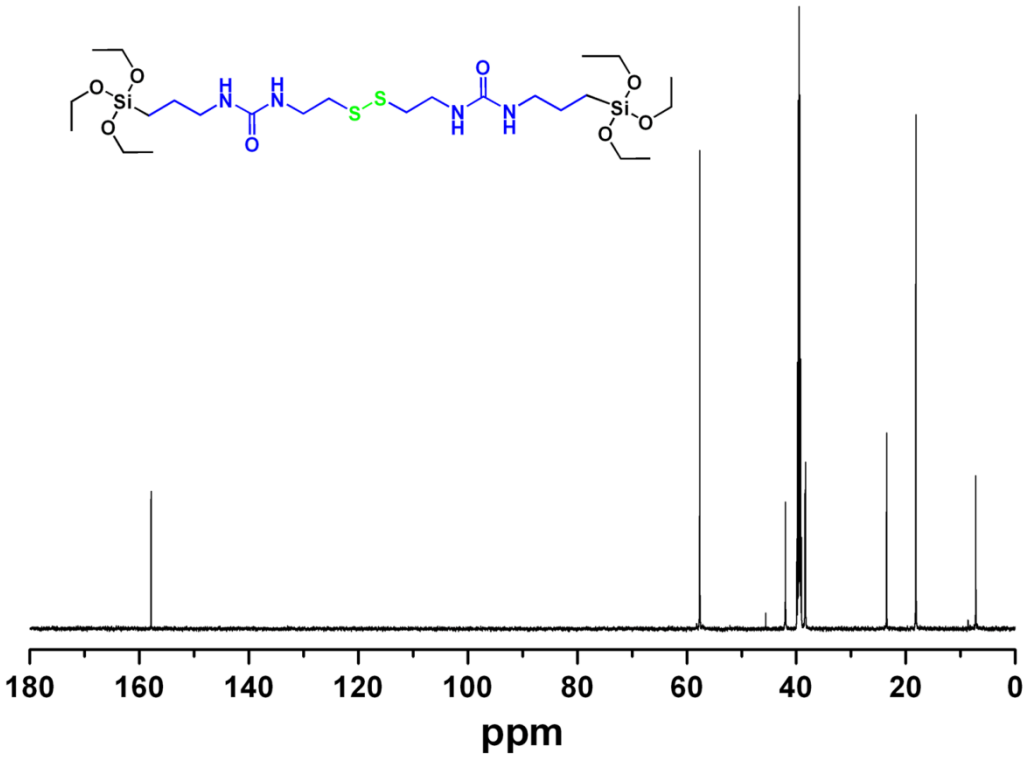

Figure S2 ^{13}C NMR spectra of disulfide bond bridged silane (BTOCD)

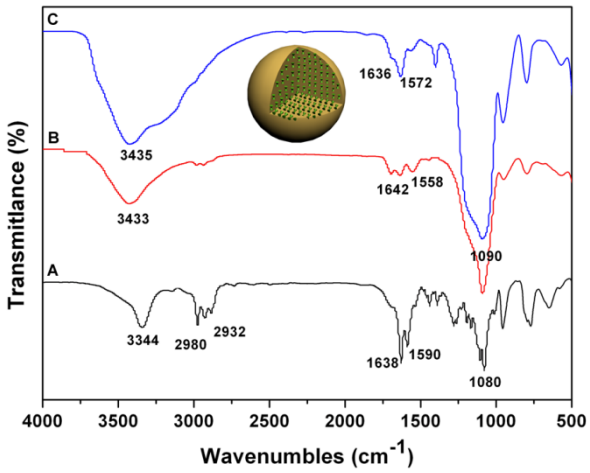

Figure S3 FT-IR spectra of BTOCD (A), silica NPs-50 (B) and silica NPs-200 (C)

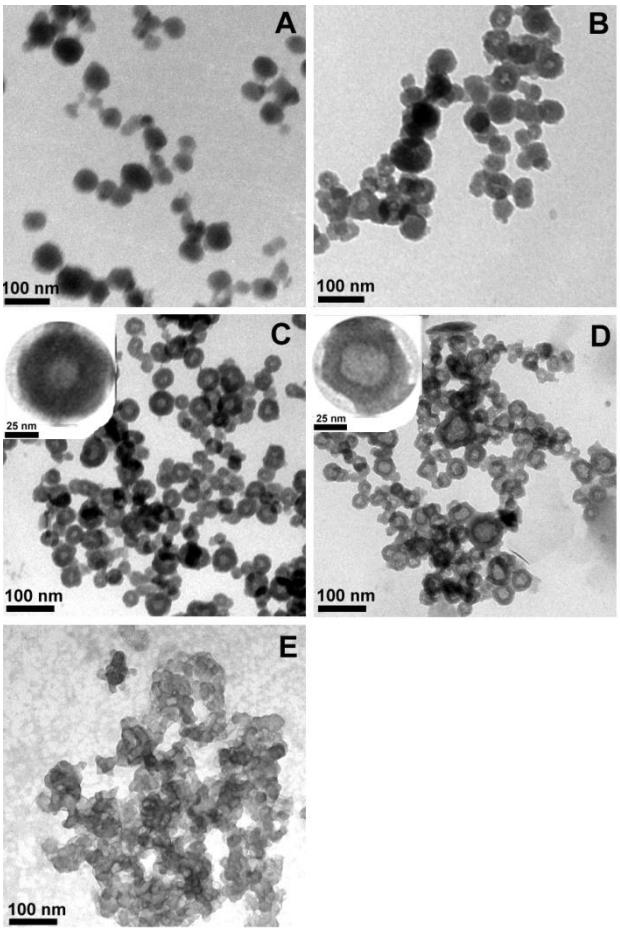

Fig.S4 TEM images of silica NPs-50 in ethanol (A), PBS (pH 7.4) without GSH (B, 3 days), PBS (pH 7.4) with GSH (C, 1 day), PBS (pH 7.4) with GSH (D, 3 days) and PBS (pH 7.4) with GSH (E, 5 days)

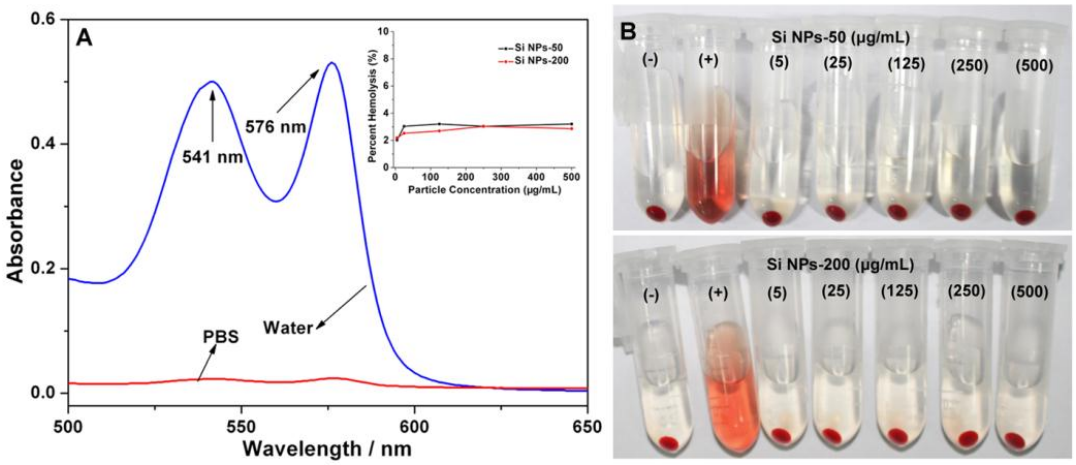

Figure S5 (A) Representative absorption spectra in the supernatant of D.I.water (Positive controls) and PBS (Negative controls). Insert on the right was the hemolysis percentages of Silica NPs-50 and Silica NPs-200 in PBS at concentrations of 5, 25, 125, 250 and 500 μg/mL. (B) Photograph of hemolysis assay to detect the presence of hemoglobin in the supernatant of Silica NPs-50 and Silica NPs-200 in PBS at above concentrations.

Table S1 Reaction conditions of size-controlled silica nanoparticles

Entry	Molar concentration (mol/L)				Average Diameter (nm)	Reaction time (h)	r/min
	TEOS	BTOCD	$NH_3 \cdot H_2O$	Water			
1	0.165	0.017	1.03	2.22	50	12	400
2	0.348	0.017	2.04	4.44	200	4	250

References

1 G. L. Li, J. Y. Liu, Y. Pang, R. B. Wang, L. M. Mao, D. Y. Yan, X. Y. Zhu, J. Sun, *Biomacromolecules*, 2011, 12, 2016.

2 Z. G. Xu, D. D. Wang, M. Guan, X. Y. Liu, Y. J.Yang, D. F. Wei, H. X. Zhang, *ACS Appl. Mater. Interfaces*, 2012, 4, 3424.