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Antibacterial properties of nitric oxide-releasing porous silicon nanoparticles

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Synthesis and characterization of GSNO

To synthesize GSNO, GSH (0.976 mmole), HCl (0.1 mL, 36%) and DI water (2 mL) were mixed and kept in the dark at 5°C. Next, cold $NaNO_2$ (1.45 mmole; 1 mL D.I. water) was dropwise added into the mixture and then was left for 30 min in order to complete the reaction. Subsequently, the synthesized GSNO was precipitated through the use of acetone/water mixture (4/1 v:v) and acetone (at 5°C). To remove unreacted GSH, the pink sediment was dissolved in cold water (5°C), and then pure GSNO was precipitated by means of acetone/water mixture (4/1 v:v) and acetone. The GSNO sediment (62 mg) was dried and stored at -20°C. ^{1}H NMR and mass spectroscopy were performed to confirm the synthesis of GSNO.
NMR spectra were recorded through the use of a Bruker 300 MHz spectrometer. All chemical shifts were reported in ppm (δ), and referenced to the chemical shifts of residual solvent resonances.

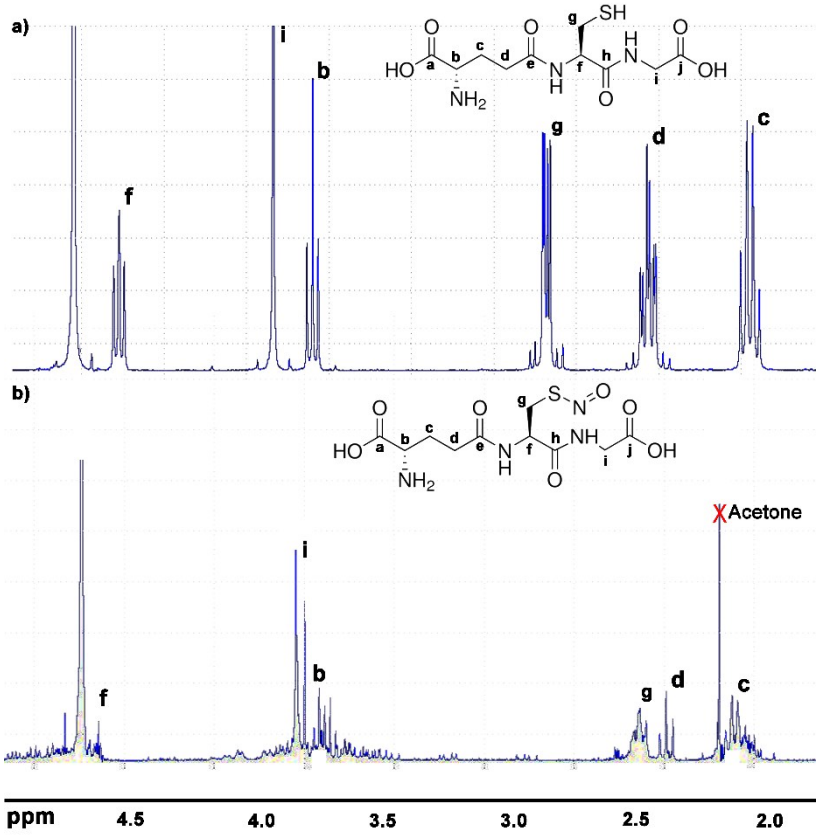

Fig. S1. ^{1}H NMR spectrum of GSH and GSNO recorded in deuterated water (300 MHz).

Time-of-flight mass spectrometry (ToF-MS) measurements were carried out using an AB Sciex TripleTOF 5600 mass spectrometer (SCIEX, Framingham, MA). All spectra were acquired in positive ion mode over the mass to charge range, m/z, with a spray voltage of 3.5 kV, a capillary voltage of 44 V, and a capillary temperature of 150°C. Nitrogen was used as sheath gas while helium was used as auxiliary gas. The sample (1mg/ml) was prepared by dissolving into deionized water containing 5% acetonitrile and 0.05% formic acid. All spectra were then recorded with a resolution of 0.1 Da.
The ToF-MS spectra showed a signal at 337.0787 Da attributed to the parent ion of GSNO, signals at 232.0455 and 307.0811 Da attributed to the GSNO fragments and also a signal at 329.0628 Da attributed to GSH (Na^+), another GSNO fragment.[1,2]

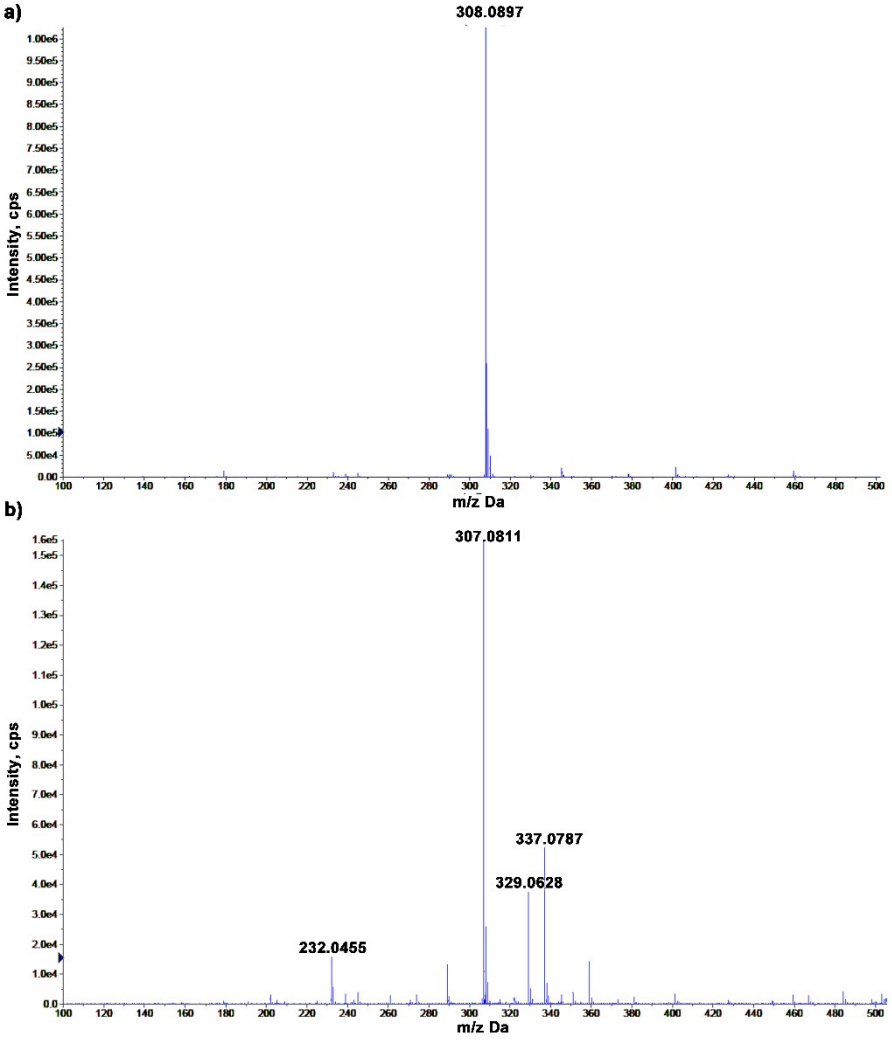

Fig. S2. ToF mass spectra of (a) GSH and (b) GSNO.

Labelling of mercapto-silane modified pSiNPs

F5M assay

Fluorescein-5-maleimide (F5M) dye (100 µg/mL) in PBS at pH 7.4 was mixed with the mercapto-silane modified pSiNPs (0.1 mg) for 2 h at room temperature with agitation. The NPs were rinsed twice with PBS, and then resuspended in PBS. Particle fluorescence was determined on a LS 55 spectrofluorometer (PerkinElmer, Waltham, MA) and on an inverted fluorescence microscope (Eclipse Ti-S, Nikon Instruments Inc., Melville, NY).

OPA assay

OPA reagent (100 µL) and allylamine (5 µL) were mixed with the mercapto-silane modified pSiNPs (0.1 mg), and then allowed to incubate in the dark for 2 h at room temperature. Next, the pSiNPs were collected by centrifugation (8000 RCF, 5 min). The collected pSiNPs were then washed twice with PBS, and then resuspended for fluorescence measurements and imaging.

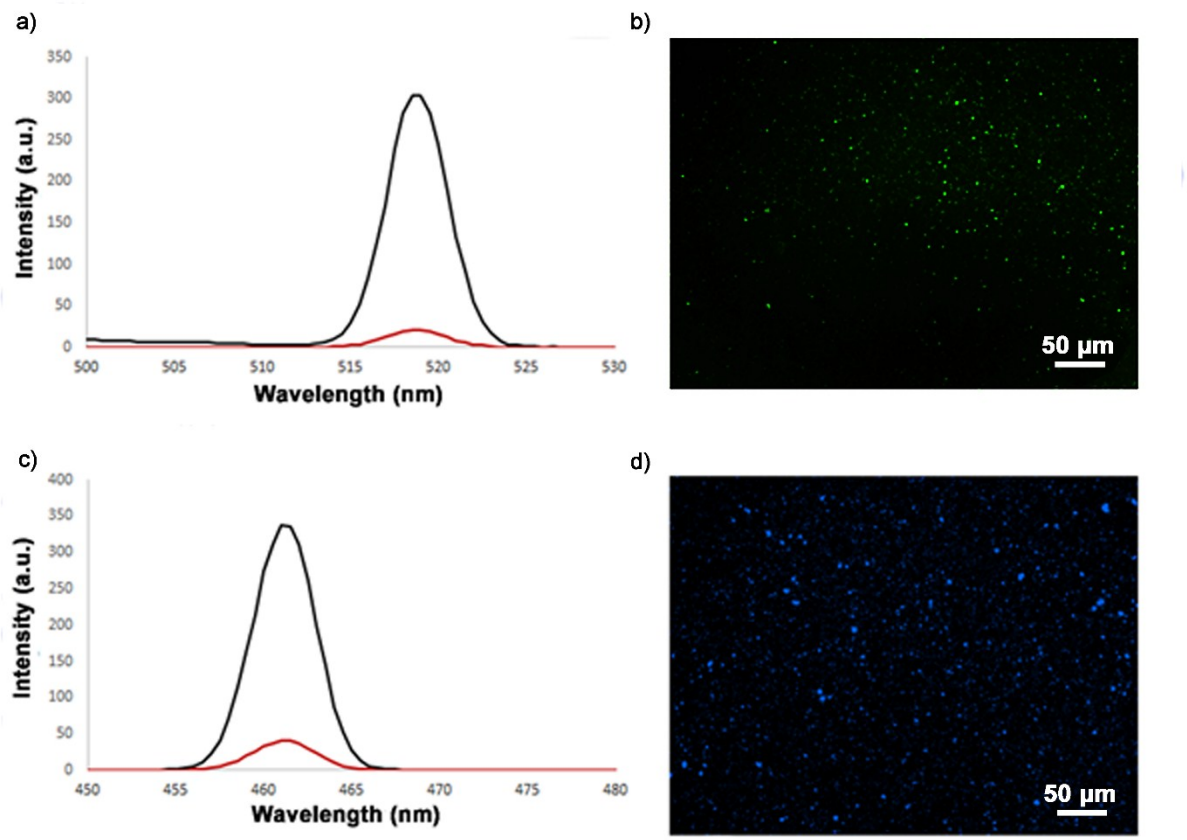

Fig. S3. Fluorescence assays of mercapto-silane modified pSiNPs. F5M assay by means of a) fluorimetry with slit widths set at 2.5 nm for both excitation (495 nm) and emission (419 nm) (pSiNPs (black line) and PBS (red line)), and a photomultiplier voltage of 775 V and b) fluorescence microscopy (green). OPA assay by means of c) fluorimetry with slit widths set at 2.5 nm for both excitation (340 nm) and emission (461 nm) (pSiNPs (black line) and PBS (red line)) and d) fluorescence microscopy (blue).

Table S1. Measured ζ-potential of the NO-releasing pSiNPs by mean of DLS	
Sample	**ζ-potential (mV)**
Isocyanato-silane modified pSiNPs	+ 41.9
GSNO/pSiNPs	+ 32
Mercapto-silane modified pSiNPs	-10.1
SNO/pSiNPs	-33.2

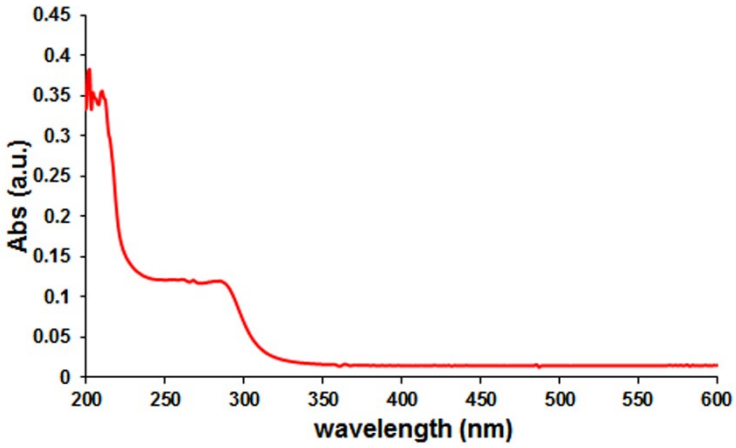

Fig. S4. UV absorbance of GSH/pSiNPs (concentration = 100 µg/mL in MeOH) as negative control.

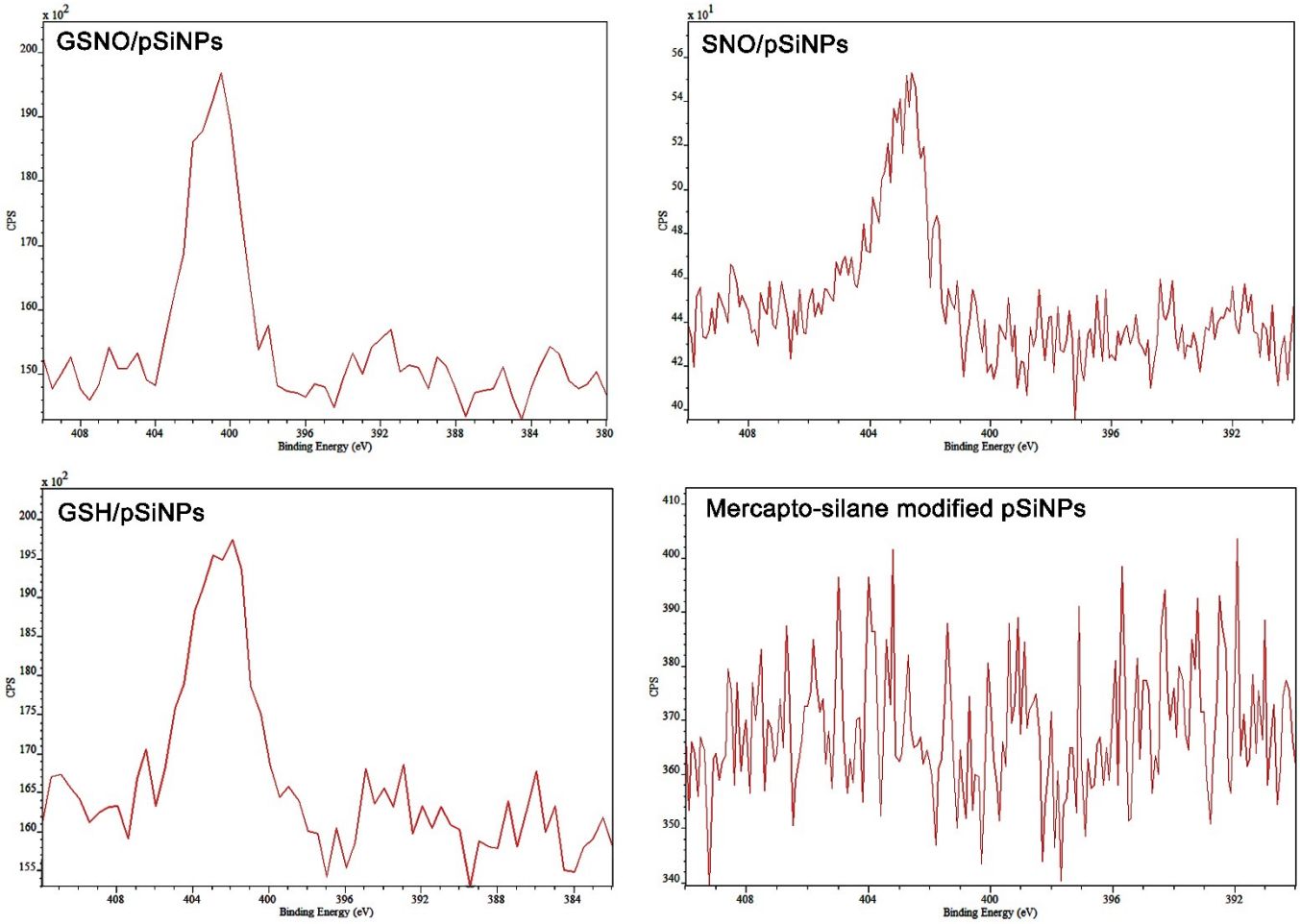

Fig. S5. High-resolution XPS spectra of N1s peaks for GSNO/pSiNPs, GSH/pSiNPs, SNO/pSiNPs and mercapto-silane modified pSiNPs.

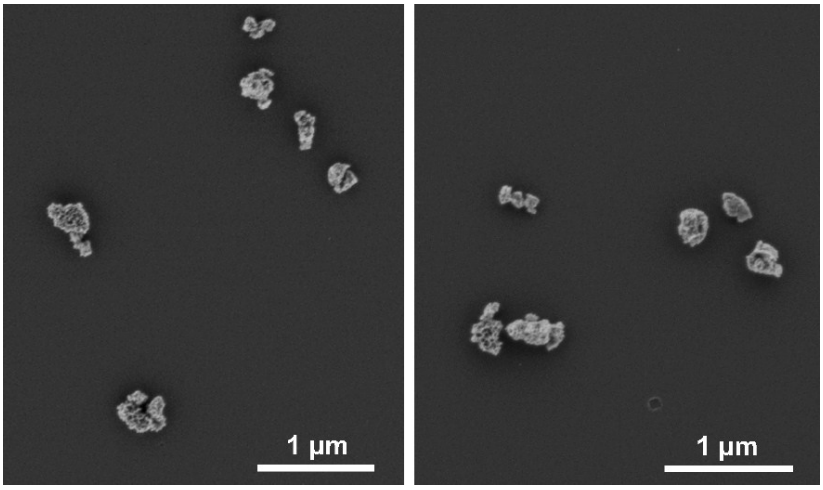

Fig. S6. Representative SEM images of the OX-pSiNPs.

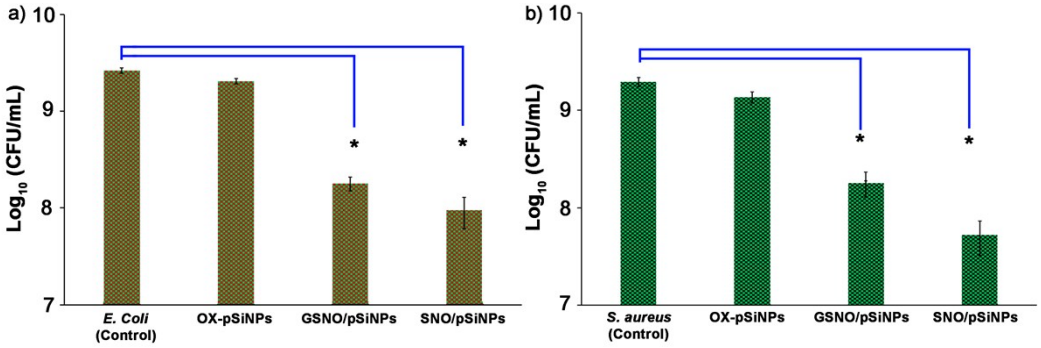

Fig. S7. Inhibitory effect of NO-releasing pSiNPs (7 mg/mL) on (a) *E. coli* and (b) *S. aureus* after 24 h incubation in TSB medium without ascorbic acid (37°C, initial bacteria density 10^4 CFU/mL). The controls are TSB medium (0 mg/mL of the NO-releasing pSiNPs) contains bacteria (104 CFU/mL). Statistically significant inhibition as compared with untreated control is marked (*P < 0.001). n = 3; Mean ± standard deviation shown.

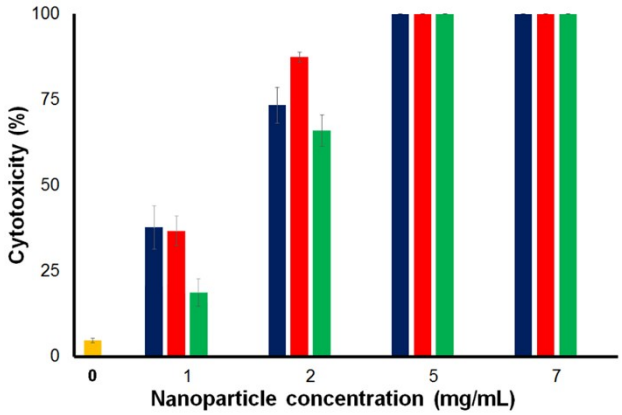

Fig. S8. Cytotoxicity assessment (via LDH assay) of NIH/3T3 fibroblasts after 24 h incubation with NO releasing pSiNPs: medium only control (yellow column), SNO/pSiNPs, (blue column) GSNO/pSiNPs (red column) and OX-pSiNPs (green column). n = 3; mean ± standard deviation shown.