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Supporting Information

Lectin-Gated, Mesoporous, Photofunctionalized Glyconanoparticles for Glutathione-Responsive Drug Delivery

Juan Zhou,[a] Nanjing Hao,[b] Thareendra De Zoyza,[b] Mingdi Yan*[a,b] and Olof Ramström*[a]

[a] Department of Chemistry, KTH - Royal Institute of Technology, Teknikringen 30, S-10044 Stockholm, Sweden; E-mail: ramstrom@kth.se
[b] Department of Chemistry, University of Massachusetts Lowell, 1 University Avenue, Lowell, MA 01854, USA; E-mail: Mingdi_Yan@uml.edu

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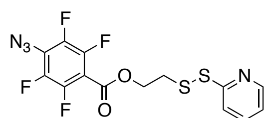
1. Experimental Section

1.1 Materials

Fluorescin isothiocyanate (FITC) and (3-aminopropyl)triethoxysilane (APTES) were purchased from TCI. Hexadecyltrimethylammonium bromide (CTAB) was purchased from Fluka. Tetraethyl orthosilicate (TEOS), (3-mercaptopropyl)trimethoxysilane, Concanavalin A (Con A) and L-glutathione (GSH) were from Sigma-Aldrich. D-(+)-Mannose was from Alfa Aesar. Doxorubicin hydrochloride (DOX) was from Apollo Scientific. Primary lung epithelial cells (PCC cells), human lung carcinoma epithelial cells (A549 cells), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin solution were purchased from ATCC. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{19}F NMR (376 MHz) were recorded on a Bruker Advance spectrometer. Transmission electron microscopy (TEM) images were obtained using a Phillips EM-400T TEM microscope. Nitrogen adsorption-desorption isotherms were peformed using a Micromeritics ASAP2010 at 77.3 K under continuous adsorption conditions. FT-IR spectra were measured on a Bio Rad FTS-375 spectrometer. Dynamic light scattering was measured with a Nanozeta (zetasizer) instrument. UV absorbance spectra were recorded using a Cary 300Bio spectrophotometer.

Fluorescence images were analyzed by a Cary Eclipse fluorescence spectrophotometer. Confocal fluorescence images were taken on an Olympus FV300 microscope.

1.2 Synthesis of 2-(pyridin-2-yldisulfanyl)ethyl 4-azido-2,3,5,6-tetrafluorobenzoate



To a solution of 2,2'-dithiopyridine (2.4 g, 11.1 mmol) in methanol (6.7 mL) was added a solution of 2-mercaptoethanol (580 mg, 7.4 mmol) in methanol (3.3 mL), the reaction mixture was stirred for 3 h at room temperature. The solvent was removed by rotary evaporation and the crude product was purified by silica chromatography. The purified product (187 mg, 1 mmol), DMAP (12 mg, 0.1 mmol) and EDC·HCl (211 mg, 1.1 mmol) were added to a solution of 4-azido-2,3,5,6-tetrafluorobenzoic acid in DCM (1.5 mL) at 0 °C, and then the mixture was allowed to warm to room temperature. After 18 h, purification of the mixture by silica chromatography provided the purified product (184 mg, 46%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.46 (d, *J* = 3.6 Hz, 1H, CH), 7.66 (d, *J* = 6.4 Hz, 1H, CH), 7.62 (m, 1H, CH), 7.09 (t, *J* = 4.8 Hz, 1H, CH), 4.61 (t, *J* = 5.2 Hz, 2H, CH_2), 3.15 (t, *J* = 5.2 Hz, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 159.4, 159.0, 149.8, 146.5, 144.4, 141.5, 139.50, 137.0, 123.7, 121.0, 119.9, 107.3, 64.2, 37.0; ^{19}F NMR (376 MHz, $CDCl_3$, ppm): δ -150.70, -138.12; IR (Figure S6); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{14}H_8F_4N_4O_2S_2$, 405.0103; found, 405.0096.

1.3 Synthesis of FITC-doped particles (FMSN particles)

Fluorescin isothiocyanate (FITC, 2.7 mg, 7 μmol) was dissolved in absolute ethanol (1.5 mL) and allowed to react with APTES (6 μL) in the dark for 4 h, yielding FITC-APTES solution. CTAB (0.25 g, 0.7 mmol) was dissolved in a mixture of distilled water (120 mL) and NaOH aqueous solution (2 M, 0.875 mL), followed by adjusting the solution temperature to 80 °C. TEOS (1.25 mL) and the FITC-APTES solution were added to the solution. The mixture was allowed to stir for 2 h to give rise to precipitates. The products were obtained by flitration and dried under vacuum. To remove the template CTAB, the crude particles were dispersed in acidic methanol (60 mL, 0.6 mL HCl) and refluxed overnight. The **FMSN** particles (237 mg) were obtained by repeated filtration until no CTAB signal could be observed by FT-IR, and dried under vacuum.

1.4 Synthesis of PFPA-functionalized FMSN particles (FMSN-PFPA particles)

FMSN particles (180 mg) were dispersed in absolute ethanol (30 mL), followed by addition of (3-mercaptopropyl)triethoxysilane (500 μL). The reaction vessel was sealed and kept at 90 °C for 24 h. The formed **FMSN-SH** particles were centrifuged at 6500 rpm for 20 min, washed by ethanol three times, and dried under vacuum. The **FMSN-SH** particles (150 mg) were dispersed in dry methanol (5 mL), and 2-(pyridin-2-yldisulfanyl)ethyl 4-azido-2,3,5,6-tetrafluorobenzoate (150 mg, 0.37 mmol) was added. The mixture was stirred at room temperature overnight. The crude particles were centrifuged at 6500 rpm for 20 min and washed by methanol three times. After drying under vacuum overnight, **FMSN-PFPA** particles (138 mg, 92 wt%) were obtained.

1.5 Conjugation of D-mannose to FMSN-PFPA particles (FMSN-Man particles)

Dispersions of **FMSN-PFPA** particles in acetone (each sample 2.5 mg/mL, 1 mL) were placed in a flat-bottom dish, and an aqueous solution of D-mannose (2.5 mg/mL, 1 mL) was added. The mixture was covered with a 280 nm long-path optical filter and irradiated with a 450 W medium pressure Hg lamp for 20 min under vigorous stirring. Centrifugation of the solution at 6500 rpm for 20 min yielded the **FMSN-Man** particles. Excess carbohydrate was removed by membrane dialysis in water for 36 h, after which the **FMSN-Man** particles (62 mg, 62 wt%) were collected by centrifugation.

1.6 Preparations of DOX-loaded and Con A-gated particles (FMSN-DOX-Con A particles)

FMSN-Man particles (30 mg) were dispersed in PBS buffer (30 mL, 0.01 M, pH 7.4) containing DOX (2.5 mg), Ca^{2+} (0.1 mM) and Mn^{2+} (0.1 mM), and the mixture was stirred for 48 h at room temperature. Con A (3 mg) was added, and the mixture was further incubated for 6 h. The DOX-loaded, Con A-gated particles were collected by filtration and washed with PBS buffer containing Ca^{2+} (0.1 mM) and Mn^{2+} (0.1 mM) until no DOX leakage was observed.

1.7 GSH-responsive DOX release

FMSN-DOX-Con A particles (0.5 mg) were dispersed in PBS buffer solution (2.5 mL, 0.01 M, pH 7.4) containing Ca^{2+} (0.1 mM) and Mn^{2+} (0.1 mM), to which GSH was added, and the mixture was incubated at 37 °C. The sample was centrifuged at pre-determined time intervals and the UV absorbance of the supernatant was analyzed.

1.8 Cytotoxic studies of FMSN- and FMSN-DOX-Con A particles

FMSN- or **FMSN-DOX-Con A** particles were first dispersed in water: DMEM medium (1:4, v/v) at a concentration of 1600 μg/mL, from which concentration series were prepared from 1600 μg/mL to 3.125 μg/mL. PCC cells and A549 cells were maintained in high- and low glucose DMEM, respectively, supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37 °C with 5% CO_2 and 95% air, grown in 96 well plates at 5000 cells per well overnight (100 μL). **FMSN-** or **FMSN-DOX-Con A** particle samples (100 μL) were added to make sample concentrations from 800 μg/mL to 1.5625 μg/mL in duplicate. After 24 h, the medium was removed and washed with PBS three times, after which medium and Alamar blue dye (180 μL + 22 μL) were added and the cell cultures were incubated for 4 h. Finally, the fluorescence intensities were measured at an emission wavelength of 590 nm.

1.9 Confocal fluorescence microscopy

A549 cells and PCC cells were maintained in high- and low glucose DMEM, respectively, supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37 °C with 5% CO_2 and 95% air. For intracellular localization, 10^5 cells per well were seeded in 6-well plates equipped with a cover glass and allowed to adhere for 24 h. After incubation with 200 μg/mL of **FMSN-** or **FMSN-DOX-Con A** particles for 6 h, the cells were washed with PBS, mounted onto glass slides, and examined by confocal fluorescence microscopy.

2. Characterizations

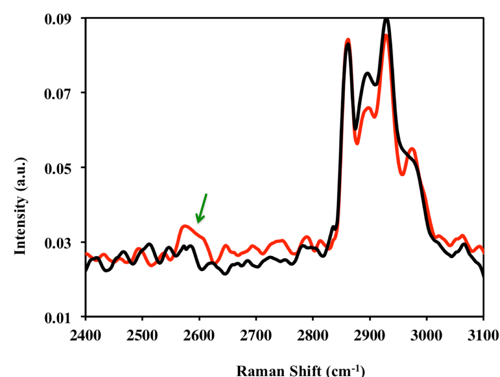


Fig. S1 Raman spectra of **FMSN-SH** particles (red) and **FMSN-PFPA** particles (black). The arrow indicates the absorption of thiol groups on **FMSN-SH** particles around 2580 cm^{-1}.

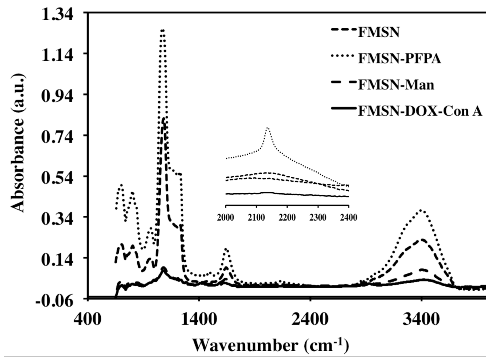


Fig. S2 FT-IR of nanoparticles.

2.1 Determination of D-mannose density and coupling yield

Theoretical maximal D-mannose density was calculated using the equation:

$$\rho_{max} = \frac{6}{\rho d S_0 N_A}$$

ρ = particle density (2.2 g/cm^3)[1]
d = particle diameter (130 × 10^{-7} cm)
S_0 = D-mannose space occupancy (0.24 × 10^{-14} cm^2)[2]
N_A = Avogadro's number
=> ρ_{max} = 1.4 × 10^{-4} mol/g
Experimental D-mannose density estimated from anthrone method (triplicate samples):[3] ρ_{exp} = 6.7 × 10^{-5} mol/g (12 mg/g).
D-Mannose coupling yield = ρ_{exp} / ρ_{max} × 100% = 48%.

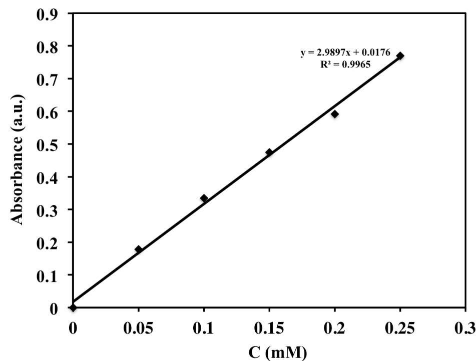


Fig. S3 Calibration curve for D-mannose treated with anthrone/H_2SO_4 solutions. Absorption wavelength 620 nm. Each measurement was repeated 3 times.

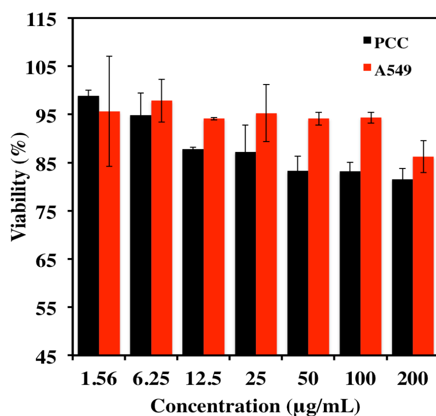


Fig. S4 Percent viability of A549 cells and PCC cells against **FMSN** particles.

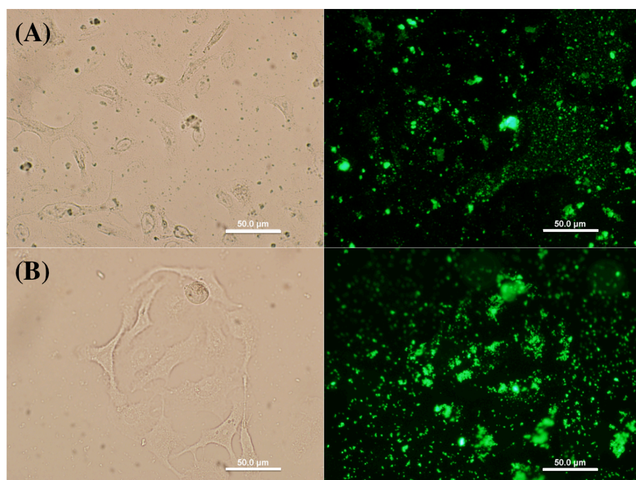


Fig. S5 Confocal microscopic images of PCC cells (A) and A549 cells (B) incubated **FMSN** particles. Scale bars: 50 μm.

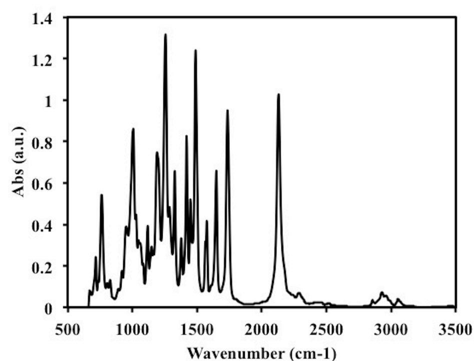


Fig. S6 FT-IR of 2-(pyridin-2-yldisulfanyl)ethyl 4-azido-2,3,5,6-tetrafluorobenzoate.

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