Supplementary Information

for

Impact of Pore-walls Ligand Assembly on the Biodegradation of Mesoporous Organosilica Nanoparticles for Controlled Drug Delivery

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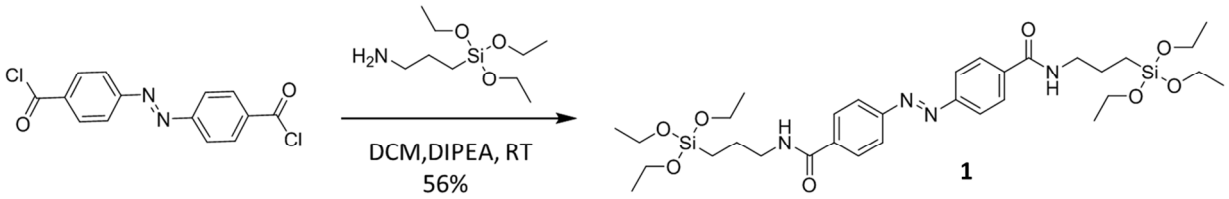
Table of Contents

1. Experimental Section

1.1. Synthesis of E-4,4'-(diazene-1,2-diyl)bis(N-(triethoxysilyl)propyl)benzamide) precursor (Azo precursor).

The precursor compound was synthesized according to the synthetic routes shown in Schemes S1. NMR spectra of compounds 1 was shown in Figures S1.



Scheme S1. Synthesis of diazotriethoxysilyl amide.

A mixture of (237 mg, 0.77 mmol) E-4,4'-(diazene-1,2-diyl) dibenzoyl chloride was dissolved in 2 ml of DCM was added to a solution of APTES (0.38 ml, 1.62 mmol) in DCM (5 ml) and DIPEA (0.30 ml, 1.70 mmol) at 0°C. After complete addition, the reaction mixture was removed from ice and stirred at room temperature for 14 h. The reaction mixture was washed with cold water and extracted with DCM (5 ml ×3) and dried over Na_2SO_4. The crude liquid was purified by column chromatography using ethyl acetate/hexane (3:1) as an eluent to yield orange solid (308 mg, 59 %). The product was dissolved in absolute ethanol (100 mg/ml) for storage and further usage. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.98 (d, J = 8.3 Hz, 2H), 7.95 (d, J = 8.1 Hz, 2H), 6.69 (br s, 1H), 3.85 (m, 12 H), 3.51 (m, 2H), 1.62 (m, 2H), 1.23 (m, 18H), 0.74 (t, J = 9.6 Hz, 2H). ^{13} C NMR (125.7 MHz, $CDCl_3$): δ (ppm) 166.6, 154.0, 137.3, 127.9, 123.3, 58.5, 42.4, 22.9, 18.5, 7.5.

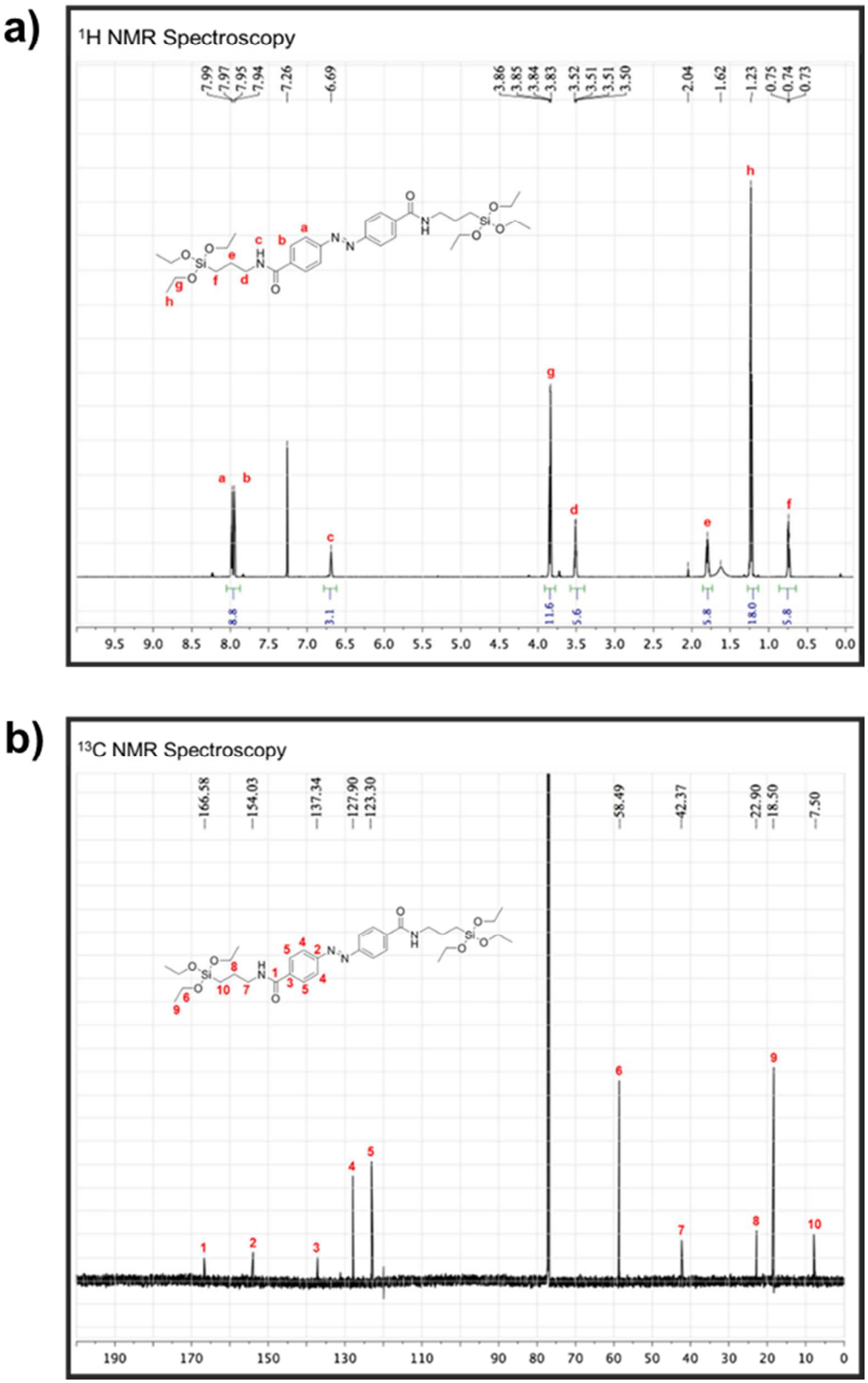
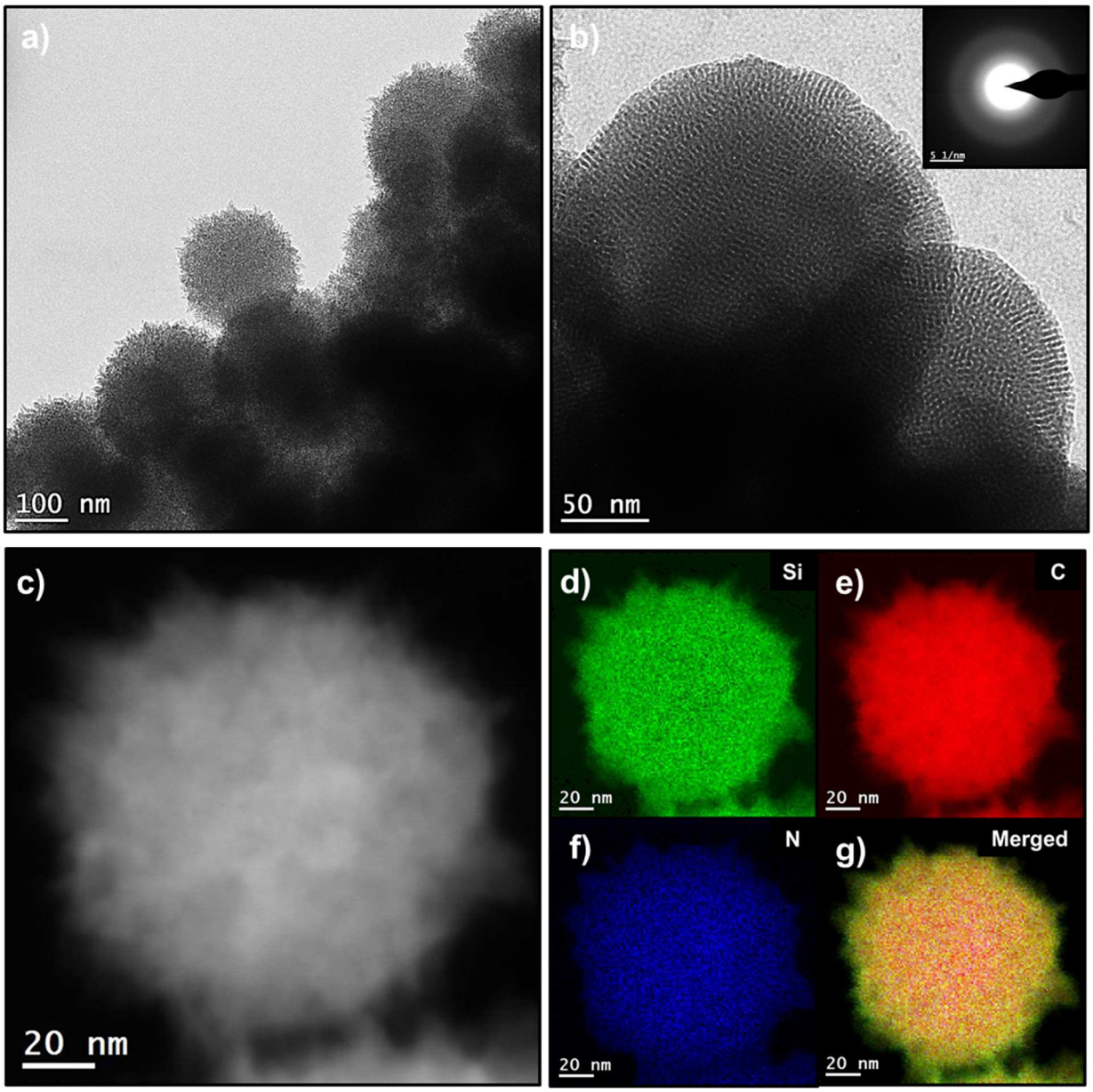
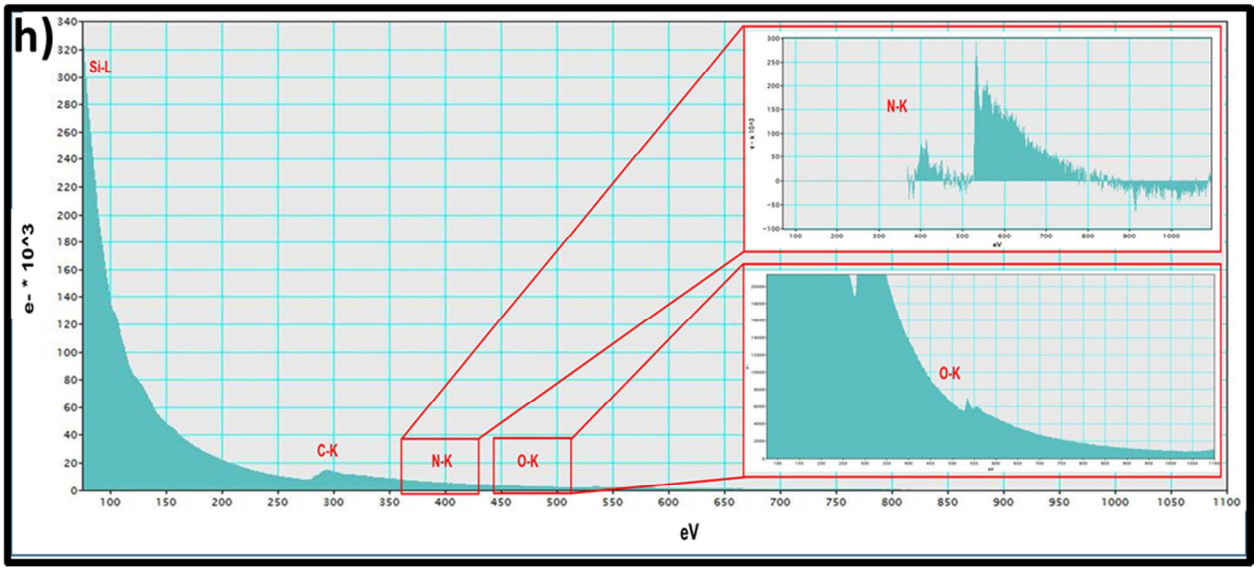


Figure S1. a) ^{1}H NMR and b)^{13}C NMR spectra of diazo triethoxysilyl amide

1.2. Supplementary TEM Image





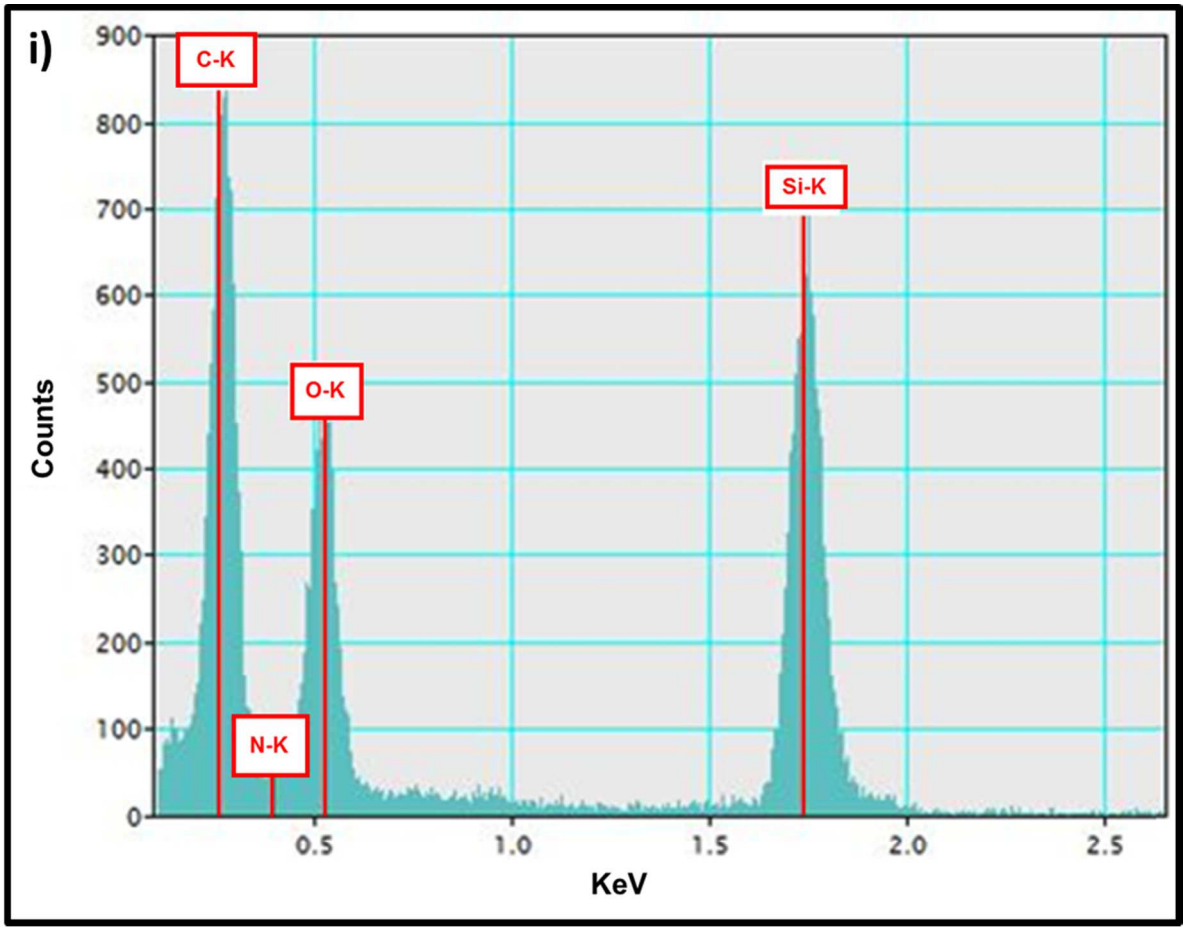
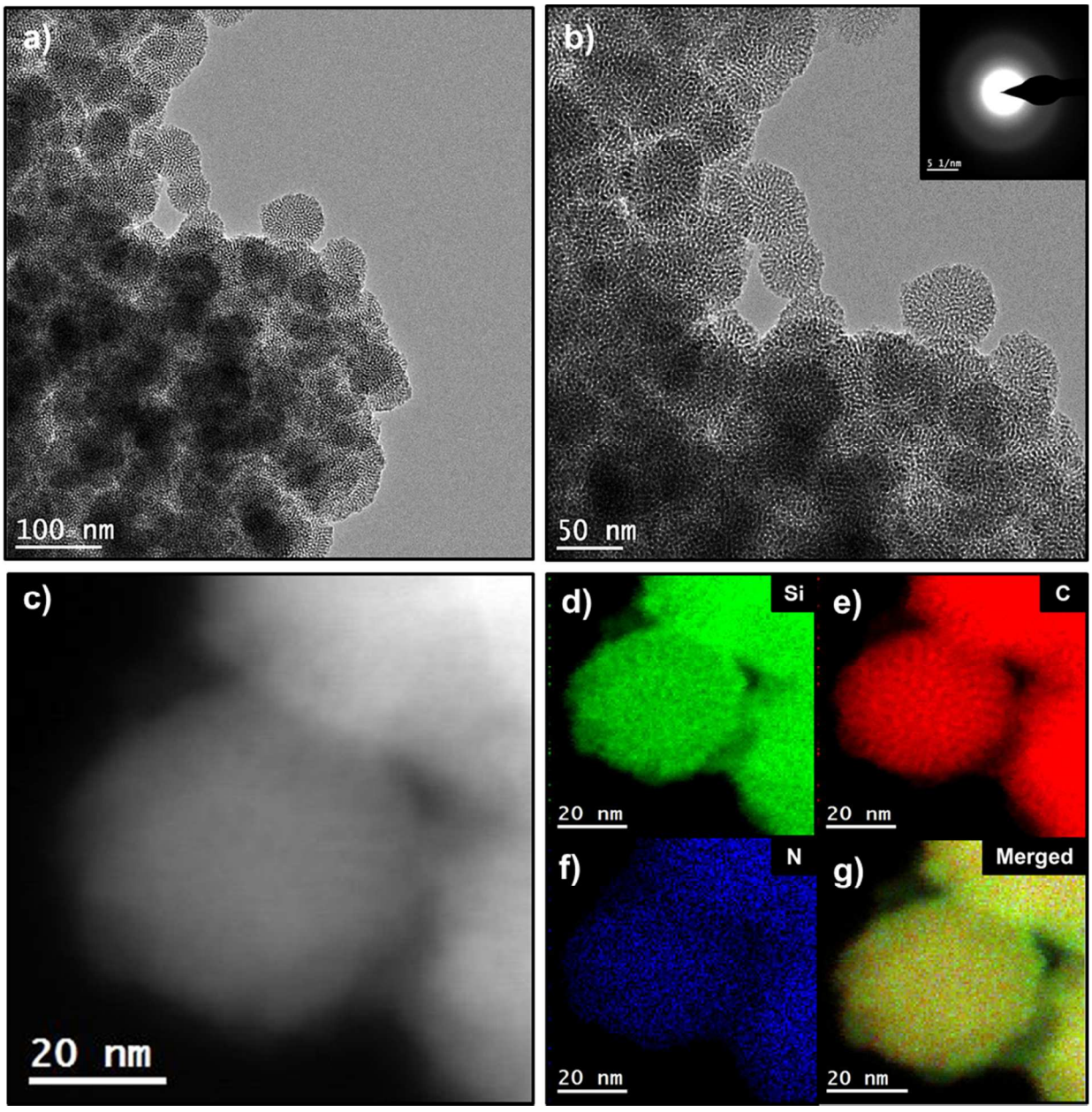


Figure S2. a) and b) TEM images of AZO-B at different resolutions. c) STEM image and (d-g)

Elemental mapping of AZO-B. h) Electron energy loss spectroscopy (EELS) of AZO-B. i) Energy Dispersive X-ray Spectroscopy (EDX) of AZO-B nanoparticles.



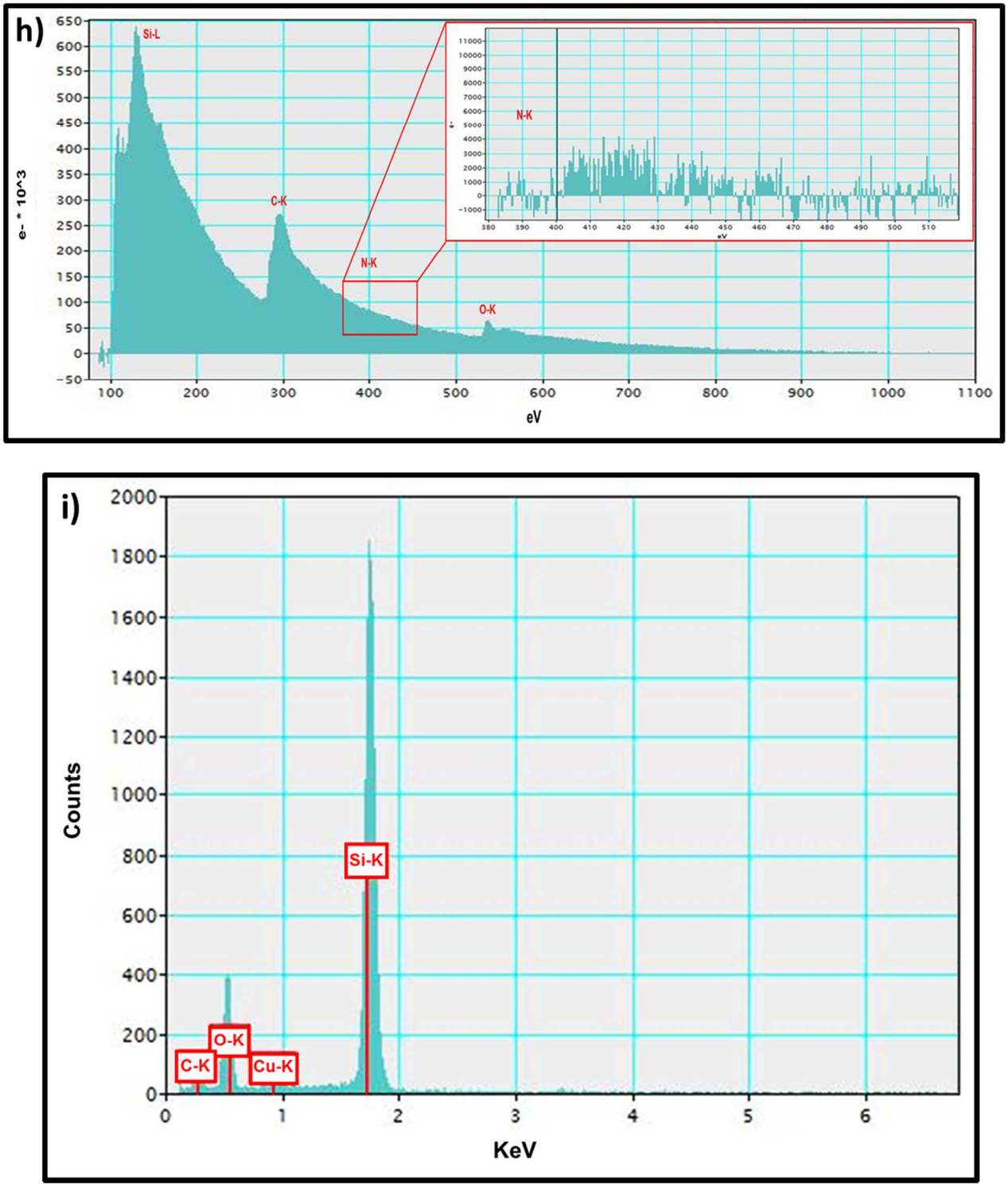


Figure S3. a) and b) TEM images of AZO-E at different resolutions. c) STEM image and (d-g) Elemental mapping of AZO-E. h) Electron energy loss spectroscopy (EELS) of AZO-E. i) Energy Dispersive X-ray Spectroscopy (EDX) of AZO-E nanoparticles.

1.3. IR Spectra

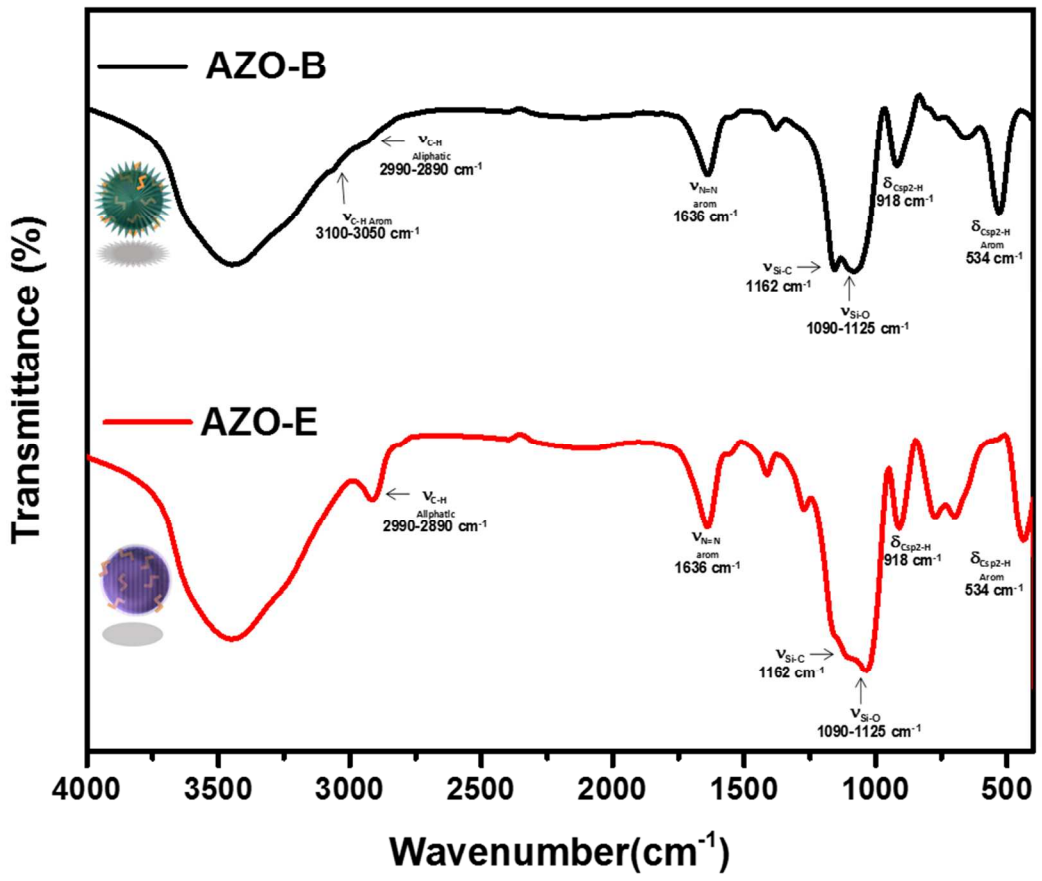


Figure S4. FT-IR spectra of AZO-B and AZO-E.

1.4. Nitrogen Adsorption–Desorption Isotherms

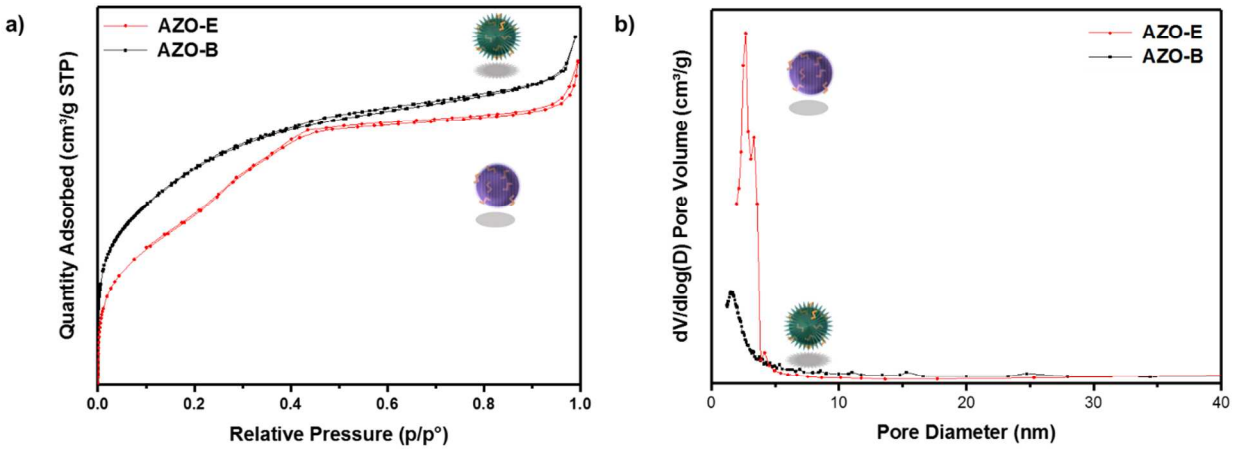


Figure S5. a) N_2 adsorption/desorption isotherms and b) pore size distribution curves of AZO-B and AZO-E.

Sample	BET surface area (m^2/g)	Total pore volume (cm^3/g)	Pore size (nm)
AZO-B	1211	0.84	1.6
AZO-E	1190	0.85	2.7

Table S1. Structural Properties of the NPs.

1.5. Nanoparticles drug loading

AZO-B / AZO-E NPs (1 mg) were mixed with doxorubicin (1 mL, 1 mg/mL) and stirred at 210 rpm for three days at room temperature. Finally, the resulted mixture was then centrifuged at 14000 rpm for 2 min and washed three times with phosphate-buffered saline solution (PBS, pH 7.4).

1.6. Enzymatically-triggered drug release

Doxorubicin-loaded nanoparticles were dispersed in a quartz cuvette with a PBS solution (300 μL, pH 7.4) to form a homogeneous solution. The particle dispersion was then mixed with azoreductase enzyme (100 μL, 1.0 mg/mL) and NADPH solution (100 μL, 25 mg/mL), and then the fluorescence spectrum was measured. The same procedure was performed for the control sample, but without adding azoreductaze enzyme and NADPH.

1.7. Nanoparticles biodegradability studies.

A mixture of AZO-B/AZO-E NPs (200 μL, 1.0 mg/mL, pH 7.4) azoreductaze enzyme (200 μL, 1.0 mg/mL) and NADPH (200 μL, 25 mg/mL) was stirred for one days at 37 °C in a centrifuge tube. Then, aliquots were directly taken to perform TEM images.

2. In Vitro Studies

2.1. Cell viability assay.

2.1.1. Materials

Colorectal carcinoma cell line (HCT-116) was obtained from American Type Culture Collection (ATCC, USA). Fetal bovine serum (FBS), Eagle's MEM medium (EMEM) and penicillin-streptomycin antibiotic were purchased from Invitrogen (USA). Cell Counting Kit-8 assay (CCK-8) was purchased from Sigma-Aldrich (USA).

2.1.2. Cell culture

HCT-116 cells were cultured in EMEM media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 humidified atmosphere. After cell detachment, the cell suspension was centrifuged, and the pallet was collected, and cells were counted for further plating. Cells were seeded in 96 well plates at a density of 7 × 103 /well. After 24h, cells were treated with different concentrations of (AZO-B/ AZO-E NPs) for 24h at 37 °C. Then, 100 μL of CCK8 solution in MEM media was added to each well after washing and incubated for 4h in darkness. The absorbance values were measured at 590 nm using the xMark™ microplate absorbance spectrophotometer.

2.2. Drug release in HCT-116 cells by confocal microscopy (CLSM)

HCT cells were seeded on coverslips placed in two 6 well plates and cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in a 5%

CO_2 humidified atmosphere. AZO-B/ AZO-E NPs were incubated with cells in both plates at a final concentration of 10 μg/ml. After 14h transfection, cells were washed three times with DPBS buffer. One plate was then treated under hypoxia for 1h; the oxygen liquid dissolved was depleted in a sealed system using 2 U/ml of glucose oxidase and 120 U/ml of catalase in culture media. The second plate was used as control under normal condition (at 37 °C in a 5% CO_2). Nuclei were stained with Hoechst 33342 according to the manufacturer's instructions. Cells were then washed again and fixed with 4% paraformaldehyde before imaged by upright CLSM.

2.2.1 Supplementary confocal images

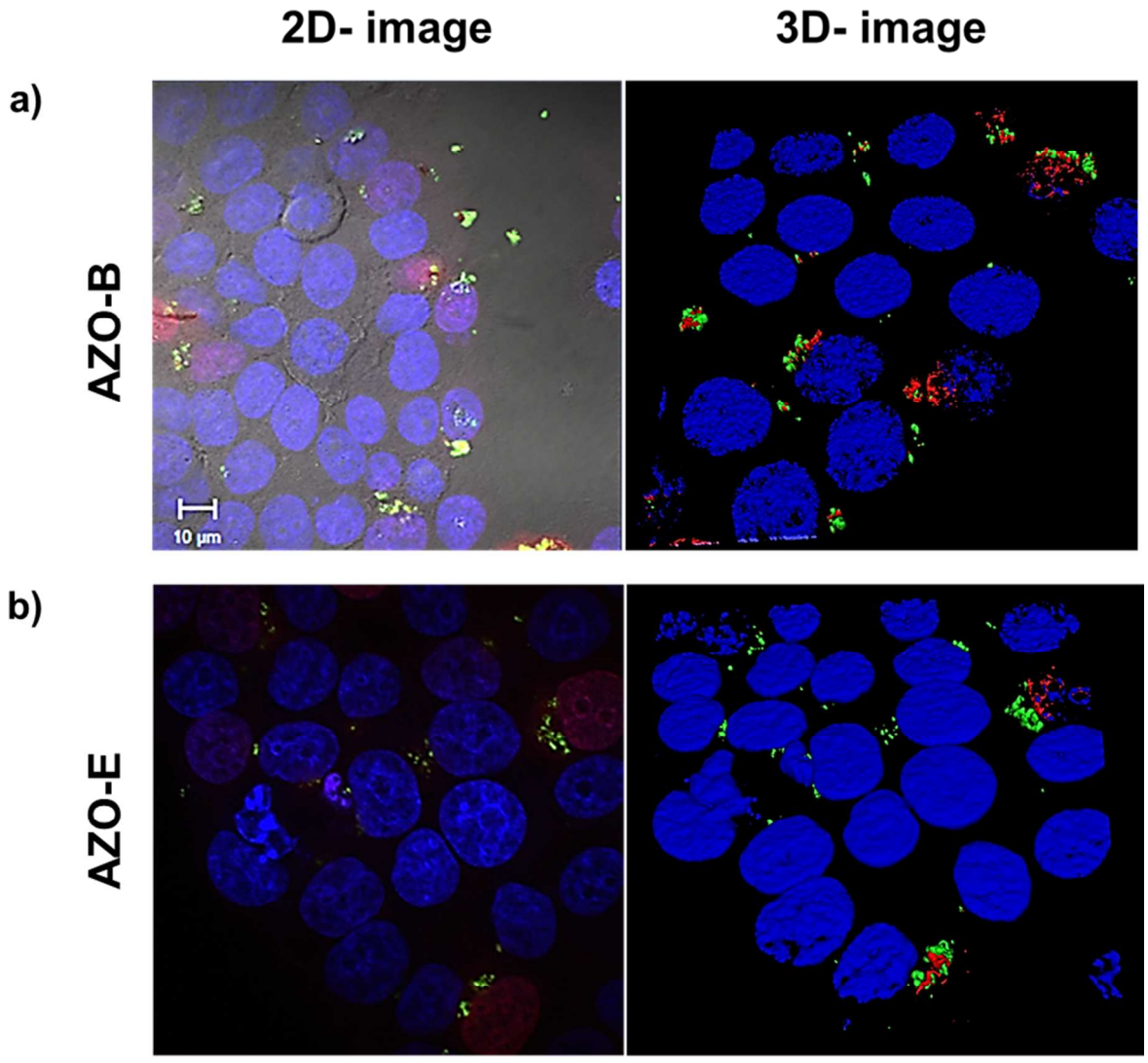


Figure S6. a) and b) 2D and 3D confocal images of HCT-116 cells incubated with DOX loaded AZO-B and AZO-E NPs under hypoxia for one hour. Nuclei are stained in blue with Hoechst 33342 dye, PMO NPs appear with the green fluorescence (FITC) and DOX fluorescence in cells (red).

3. In Vivo Studies

3.1. Materials

Freshly fertilized chicken eggs were purchased from McIntyre Farms (Lakeside, CA) and incubated in the HovaBator Genesis 1588 Egg Incubator (Incubator Warehouse). Teflon O-Rings (010 PTFE) were purchased from the O-Ring Store. 3M Tegaderm™ transparent dressing was purchased from Moore Medical. The Dremel 7700-1/15 MultiPro 7.2-Volt Cordless Rotary Tool Kit was used to make holes in the eggshell. RPMI 1640 culture medium, fetal bovine serum, penicillin/streptomycin, and Trypsin/EDTA were purchased from Life Sciences.

3.2. Methods

3.2.1 Preparation of tumors in chicken eggs

OVCAR-8 cells, an ovarian carcinoma cell line, are maintained at 37°C and 5% CO_2 in a tissue culture incubator. These cells are infected with lentivirus to express GFP. Medium for the OVCAR-8 cells is RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were passaged using 0.25% trypsin every two days.

Freshly fertilized chicken eggs are incubated at 100 ^{0}F and 60% humidity. On day 10 of development, the chorioallantoic membrane (CAM) is dropped to make a window on the eggshell.[1,2] A Teflon ring is put onto the CAM membrane which is then gently abraded with a stirring rod. $2x10^6$ OVCAR-8 ovarian cancer cells are grafted into the ring, then the window is sealed with Tegaderm film, and the eggs are incubated.[3-5]

3.2.2 Nanoparticle injection

Three days after cancer cell inoculation, established tumors are around 5 mm in diameter. Blood vessels of CAM membrane are easily observed through the shell and marked. One small square window in the eggshell is made over the marked blood vessel. This is done so as not to rupture the blood vessel. 100 µl solution containing 1 mg NPs is injected into the blood vessel through a small window. Tumor size and green fluorescence are monitored daily.

References

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