Electronic Supplementary Material (ESI) for New Journal of Chemistry.
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Electronic Supplementary Information

Dual-Functional Cyclic Peptide Switch on Mesoporous Nanocontainers for Selective CD44 Targeting and On-Off Gatekeeping Triggered by Conformational Transformation

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Experimental Section

Materials. Hexadecyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane, propargyl bromide, copper (II) sulfate, trifluoroacetic acid (TFA), ninhydrin, piperidine, doxorubicin (DOX), glutathione (GSH), acetic anhydride, triethylamine, poly(ethylene glycol) methyl ether and sodium L-ascorbate were obtained from Sigma Aldrich and used as received. N,N'-dimethylformamide (DMF), triisopropylsilane were purchased from Acros organics. 3,6-Dioxa-1,8-octane-dithiol, N,N-diisopropylcarbodiimide (DIC) were purchased from TCI. 1-Hydroxybenzotriazole (HOBt) and Rink Amide MBHA resin were purchased from Advanced Chem. Tech. Fmoc-L-Dap(N_3)-OH was purchased from IRIS Biotech GmbH. Fmoc-protected amino acids from Novabiochem were used as received. All solvents were purified using a published procedure.[1]

Transmission electron microscopy. TEM images were obtained using a Philips CM 200 instrument operated at an acceleration voltage of 120 kV. TEM samples were prepared by placing a drop of dispersed sample in distilled water (100 mg·L-1) onto a 300-mesh copper grid coated with carbon film and was dried in vacuum oven for over 3 hours.

Synthesis of DOX-loaded silica nanoparticles without peptide gatekeepers.

Surfactant was removed from silica nanoparticles four times by stirring Si-alkyne (500 mg) in 50 mL of ethanol with 2g of ammonium nitrate at 80 °C for 30 min. The resulting solid was washed thoroughly with ethanol. For DOX loading, surfactant-removed Si-alkyne (20 mg) was soaked in a DMF solution (0.2 mL) of DOX (1 mg) and stirred overnight. The resulting solid was washed rapidly with DMF and distilled water.

Fluorescence measurements. All the fluorescence measurements were performed using a Shimadzu RF-5301PC spectrofluorophotometer with an excitation wavelength of 485 nm (absorption maximum wavelength of DOX). Emission and excitation slit widths were set at 15 nm and 3 nm, respectively.

Fourier transform infrared spectroscopy. FT-IR spectra were obtained using VERTEX 80V vacuum spectrometer.

Zeta-potential analysis. Zeta-potential values were obtained using an OTSUKA Particle Size Analyzer ELS-Z2 using the dispersed samples in distilled water.

Powder X-ray diffraction. Powder X-ray diffraction analysis was performed on a Rigaku DMAX 2200V counter diffractometer with a $Cu_{K\alpha}$ radiation source (Operated at 60 kV, 60 mA).

Pore diameter analysis. Brunauer-Emmett-Teller (BET) nitrogen adsorption/desorption isotherms and Barrett-Joyner-Halenda (BJH) pore size distribution analysis were performed at 77 K on a Quantachrome instrument (ASAP 2020).

Antibodies. Antibodies against CD44, cleaved caspase-3, PARP, and β-actin were obtained from Cell Signaling Technology (Beverly, MA, USA).

Statistical Analysis. All grouped data are presented as the means ± S.E.M. Differences between groups were analyzed by ANOVA or Student's t-test using GraphPad Prism

software (GraphPad Software, Inc, La Jolla, CA, USA). All experiments were repeated in at least duplicate with triplicate technical replicates.

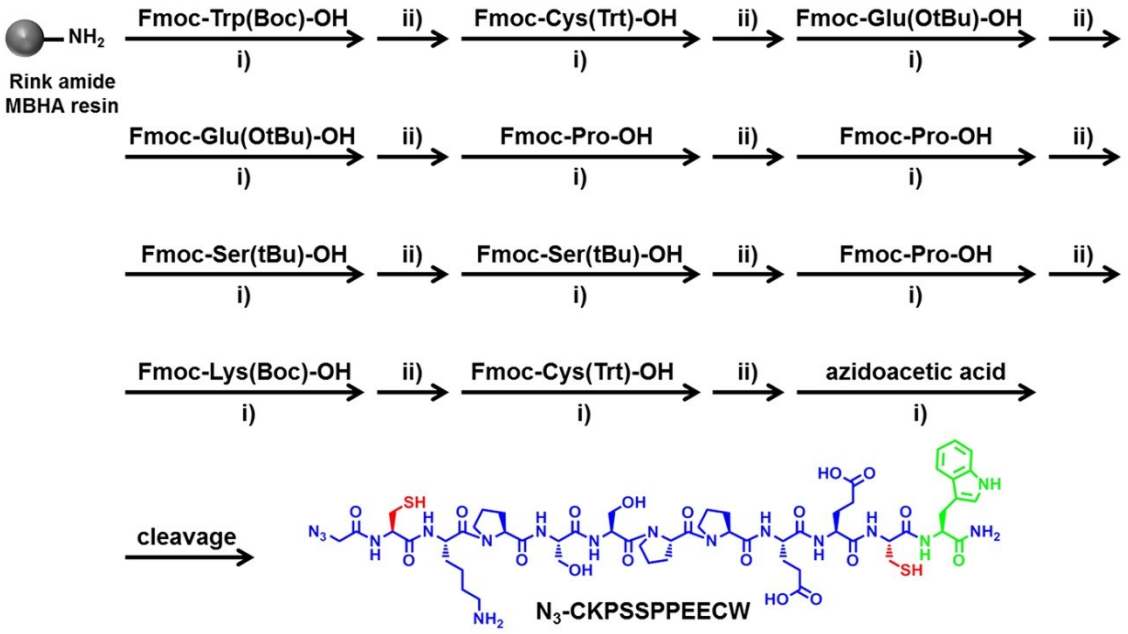

Fig. S1 Synthetic routes to N_3-CKPSSPPEECW. Conditions: i) DIC, HOBt, DMF, 4 hr; ii) 25% piperidine in DMF, 15 min.

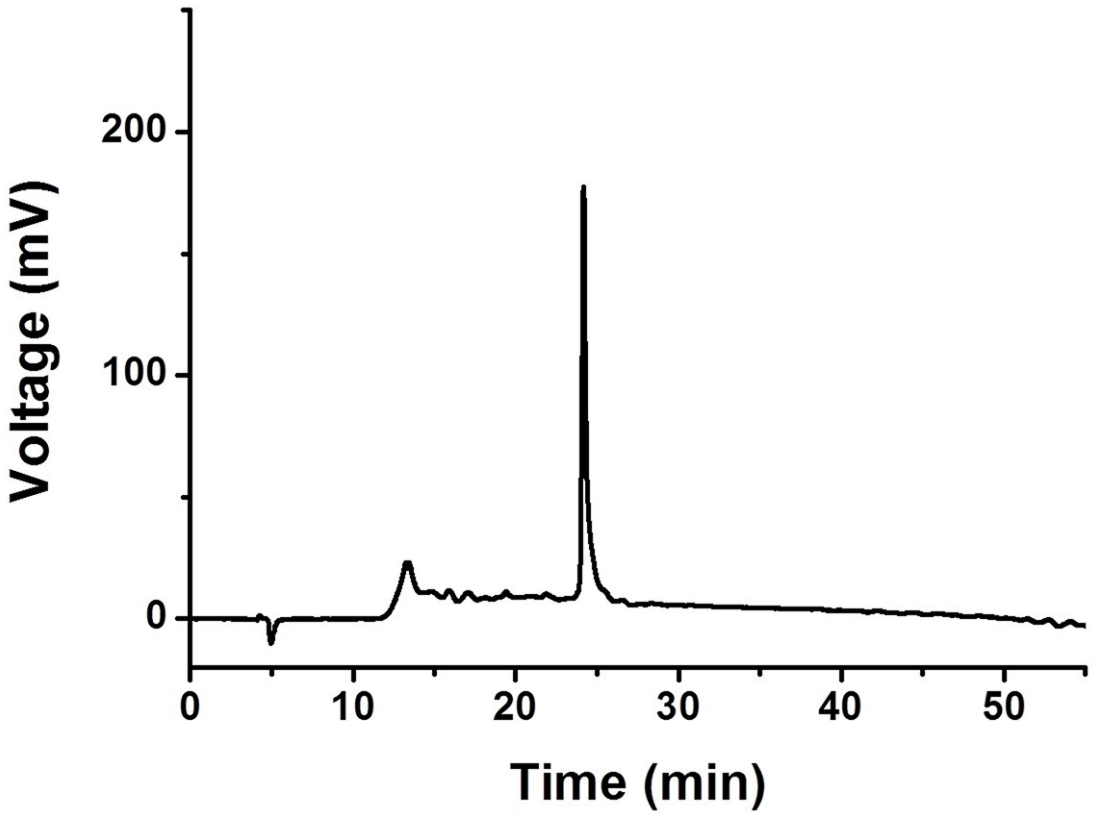

Fig. S2 HPLC chromatogram of N_3-CKPSSPPEECW.

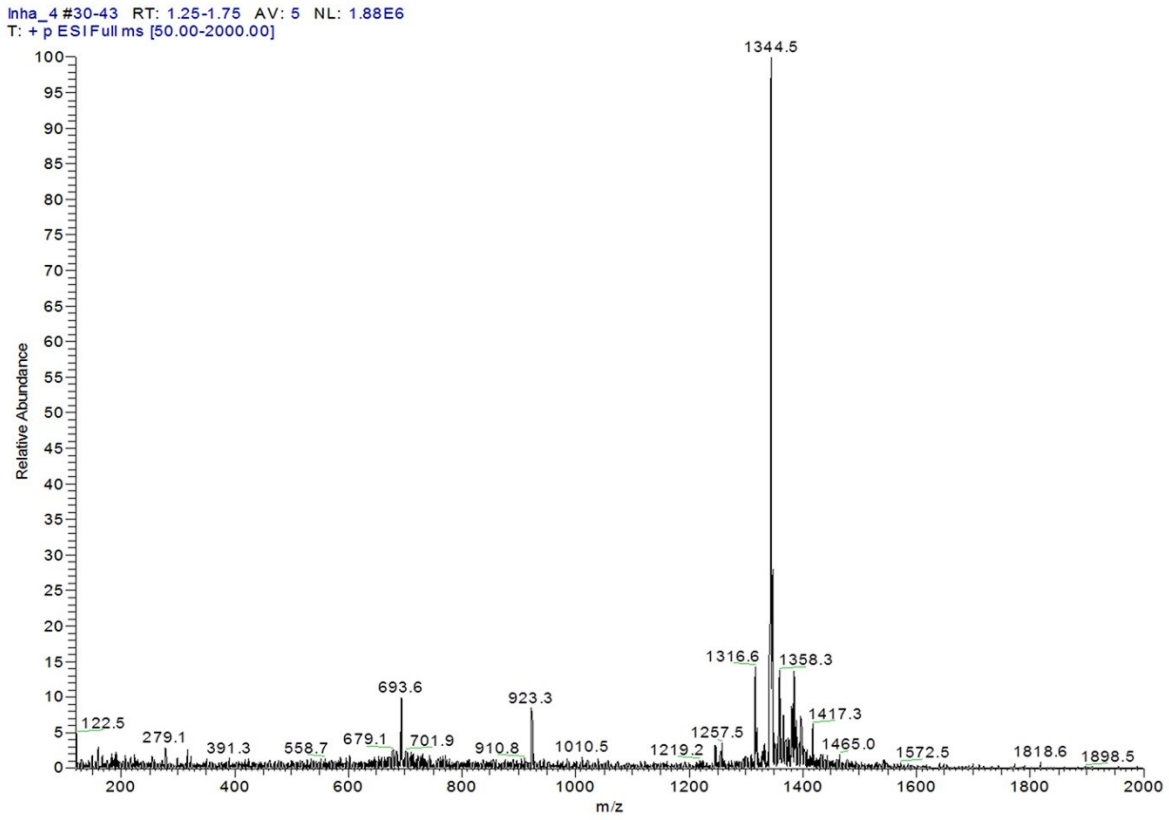

Fig. S3 ESI-Mass spectrum of N_3-CKPSSPPEECW.

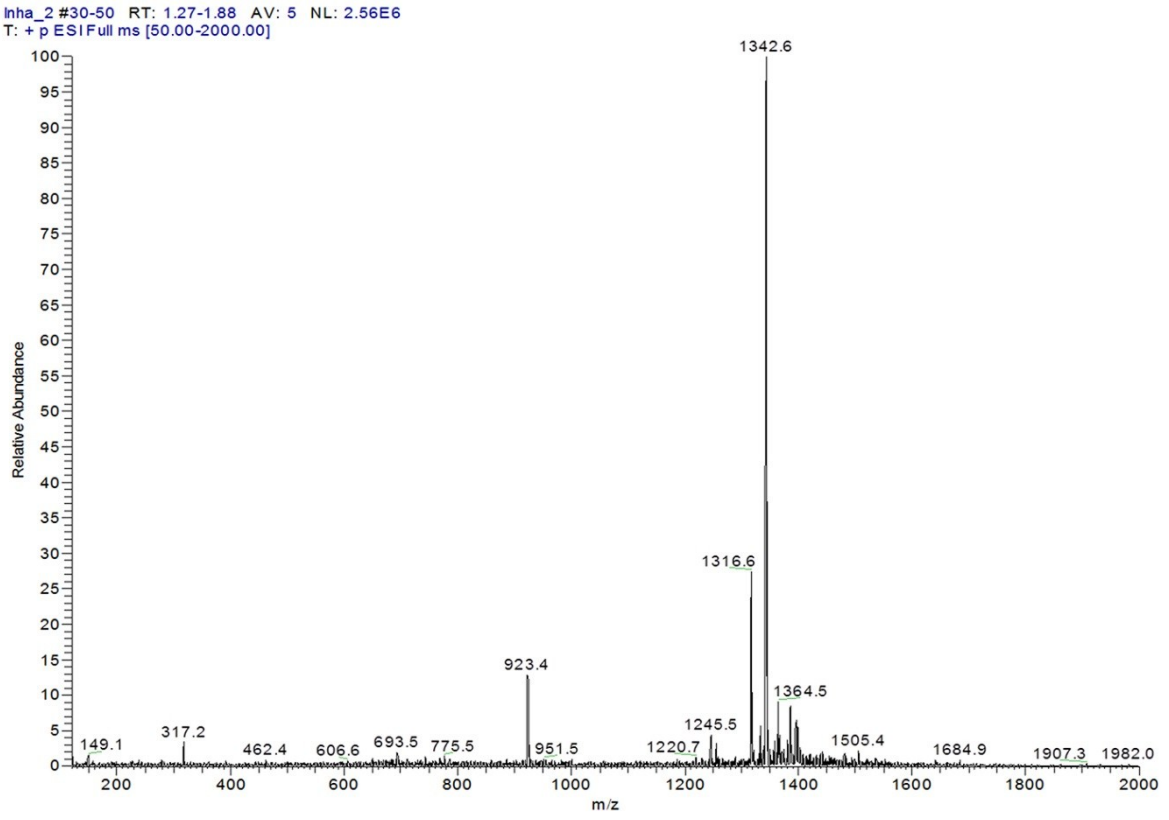

Fig. S4 ESI-Mass spectrum of N_3-CKPSSPPEECW-SS.

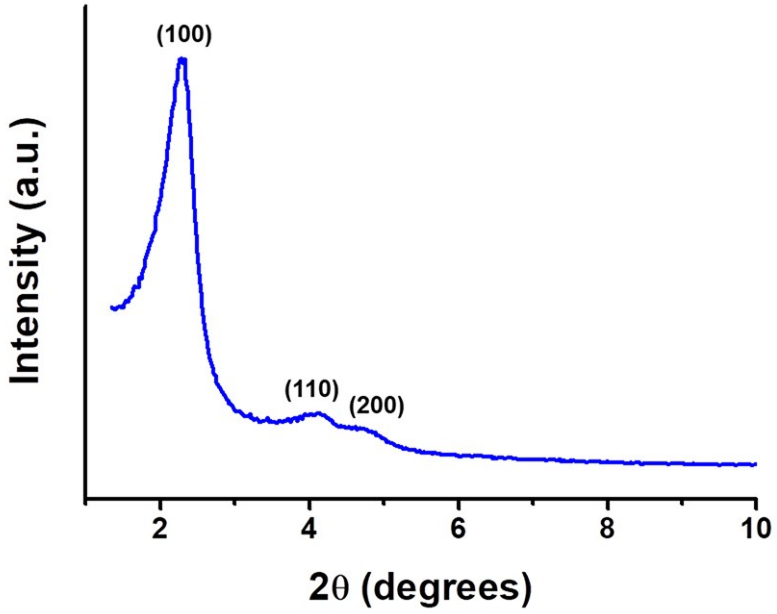

Fig. S5 Powder X-ray diffraction pattern of MCM-41.

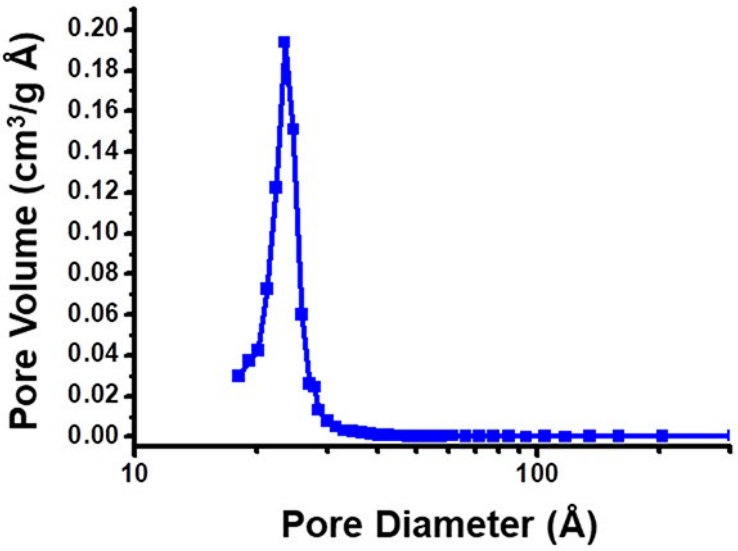

Fig. S6 Barrett-Joyner-Halenda pore size distribution analysis of MCM-41.

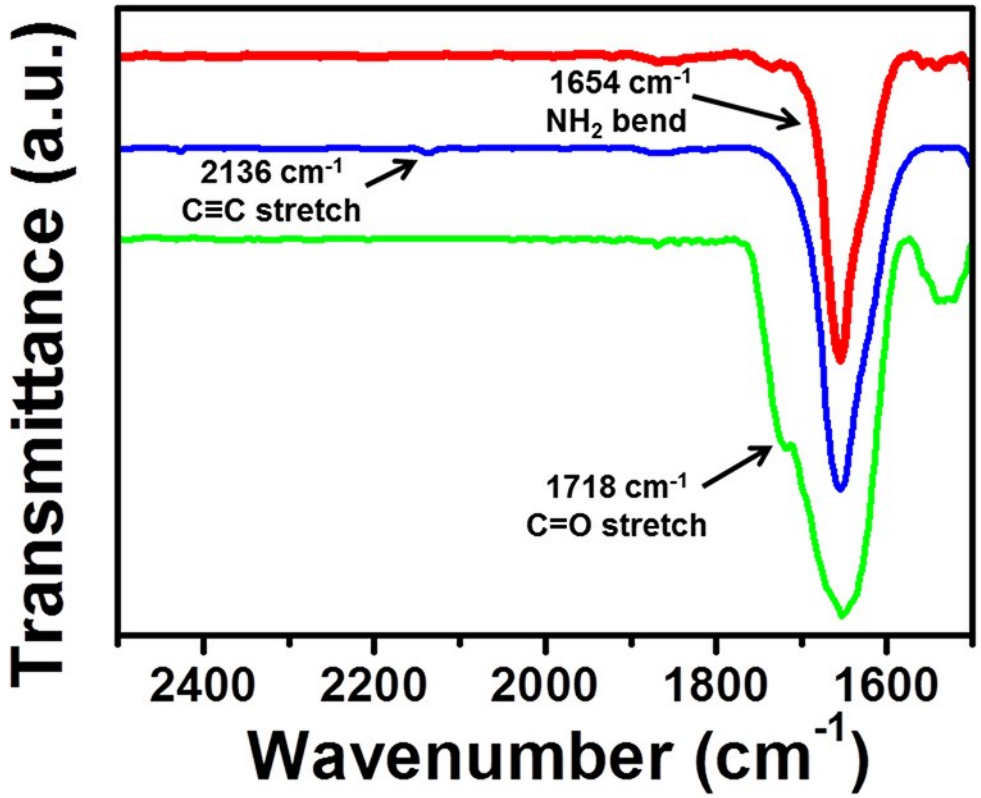

Fig. S7 FT-IR spectra of Si-NH_2 (a), Si-alkyne (b), and Si-cA6 (c).

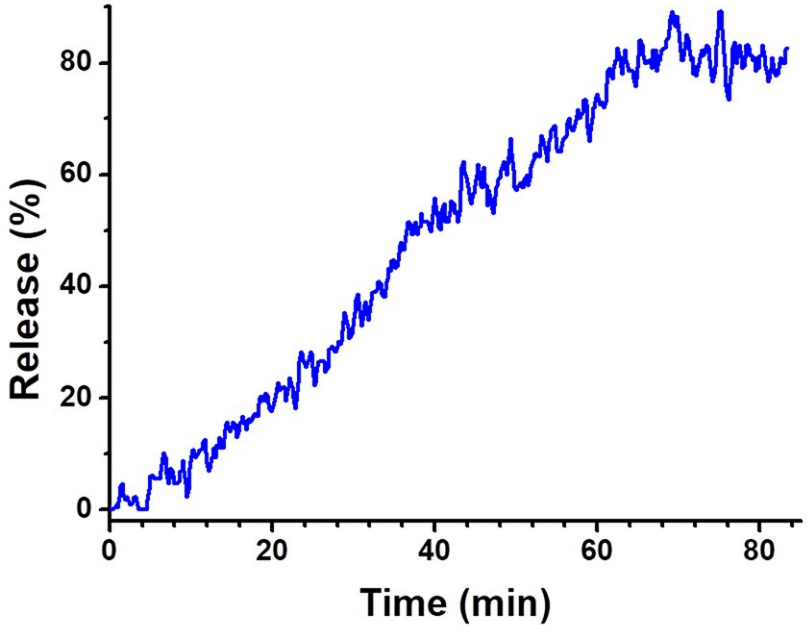

Fig. S8 Release profile of DOX from Si-alkyne without gatkeeper.

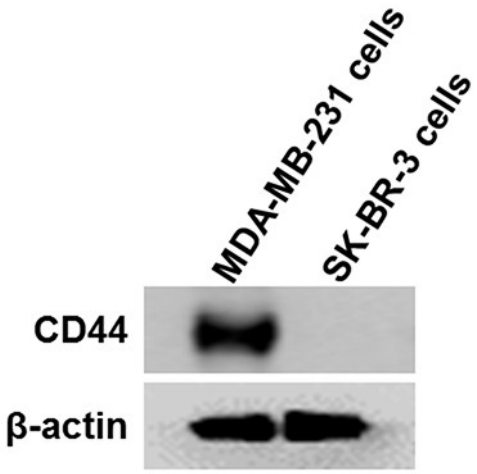

Fig. S9 Expression of CD44 in MDA-MB-231 and SK-BR-3 cells..

References

1 W. L. F. Armarego and D. D. Perrin, *Purification of Laboratory Chemicals*, Butterworth-Heinemann, Oxford, U.K, 1996.