Electronic Supplementary Information

Cyclic iRGD Peptide as a Dual-Functional On-Off Gatekeeper of Mesoporous Nanocontainers for Targeting NRP-1 and Selective Drug Release Triggered by Conformational Conversion

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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, ferric acetylacetonate 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 $mg \cdot L^{-1}$) onto a 300-mesh copper grid coated with carbon film and was dried in vacuum oven for over 3 h.

Field-emission scanning electron microscopy. FE-SEM images were obtained using Hitachi S-4200 instrument with a field emission gun at accelerating voltage of 10-15 kV and pressure range of 0.8-0.9 Torr.

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.

Thermogravimetric analysis. TGA was carried out using TA Instruments Q50 from room temperature to 500 ^{o}C at a ramp of 1 ^{o}C/min under nitrogen atmosphere.

Antibodies. Antibodies against PARP and β-actin were purchased 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.

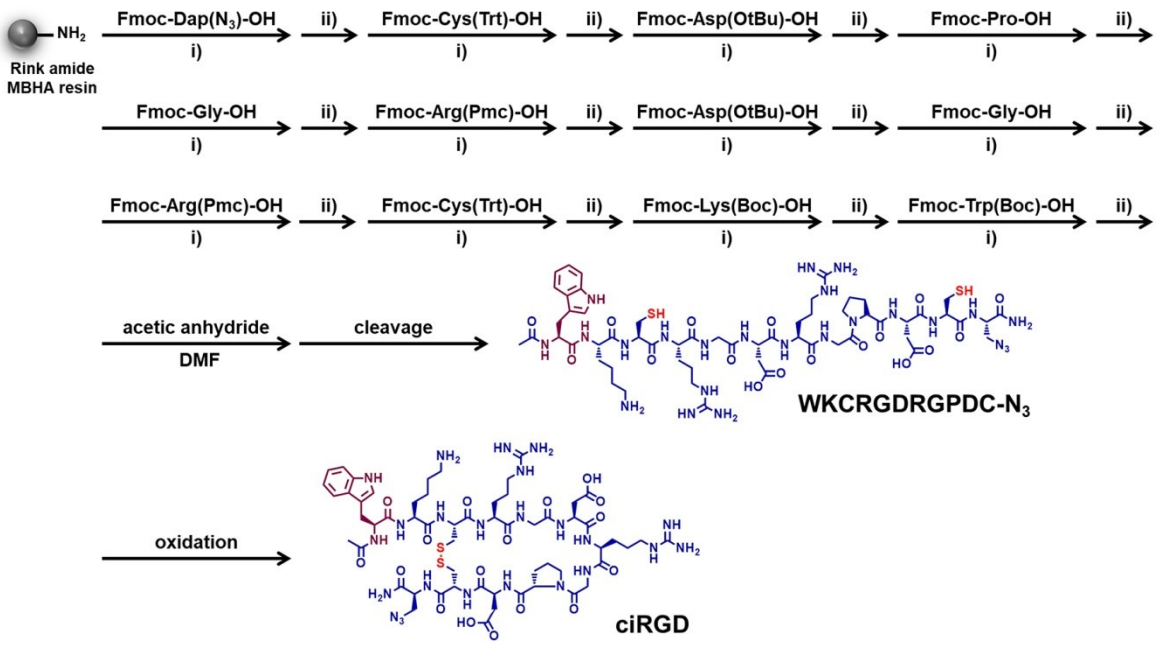

Fig. S1. Synthetic routes to WKCRGDRGPDC-N_3 and ciRGD peptides. Conditions: i) DIC, HOBt, DMF, 4 hr; ii) 25% piperidine in DMF, 15 min.

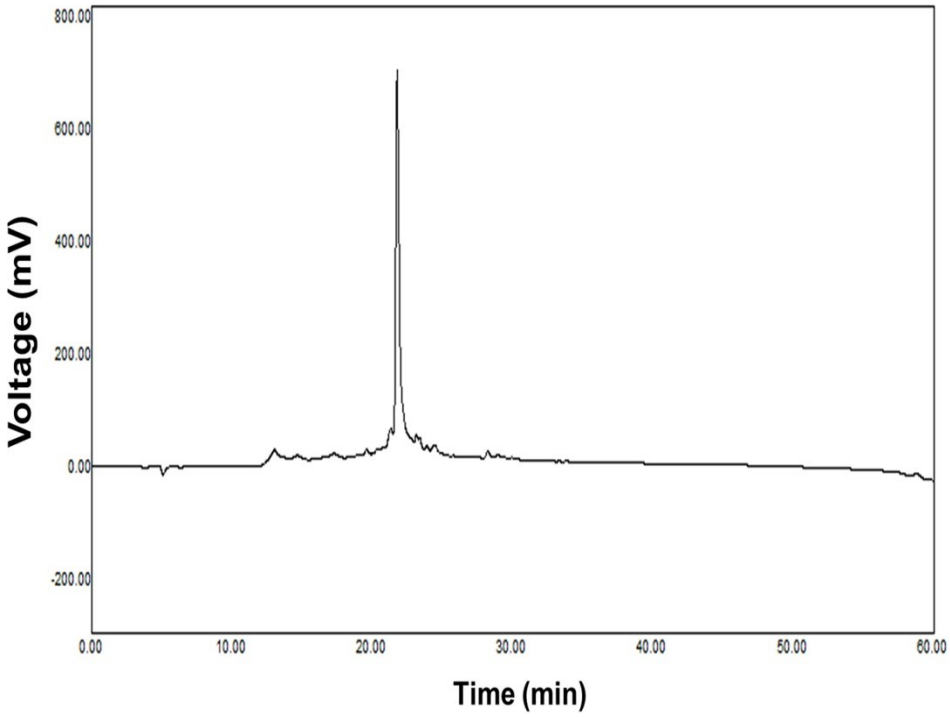

Fig. S2. HPLC chromatogram of WKCRGDRGPED-N_3.

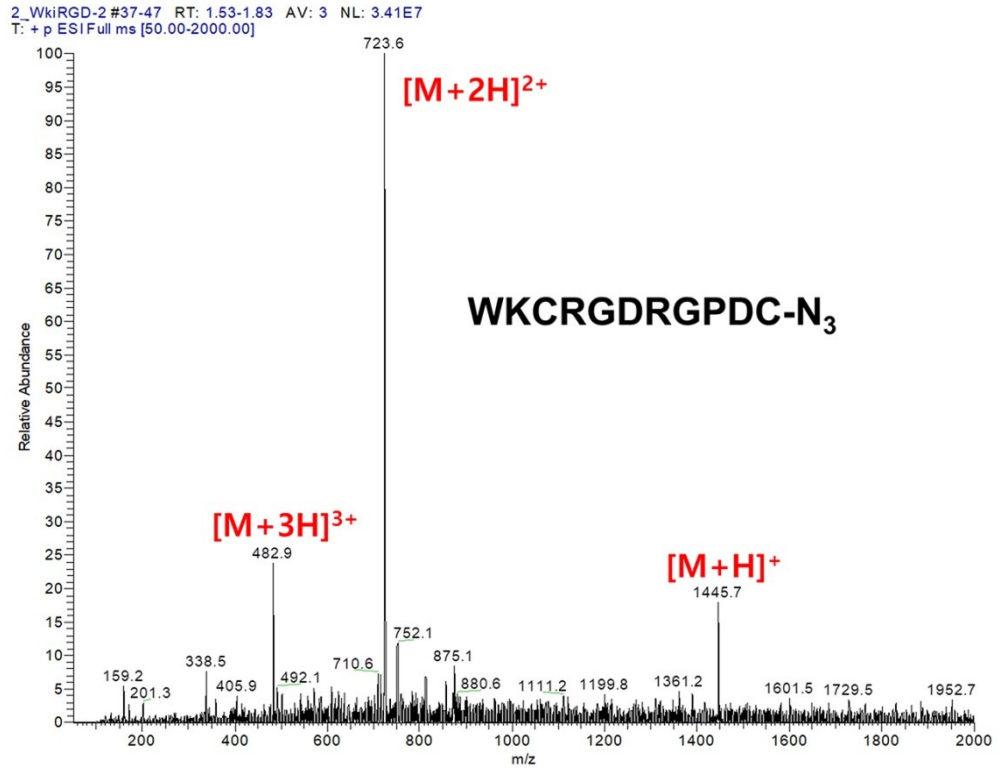

Fig. S3. ESI-Mass spectrum of WKCRGDRGPDC-N_3.

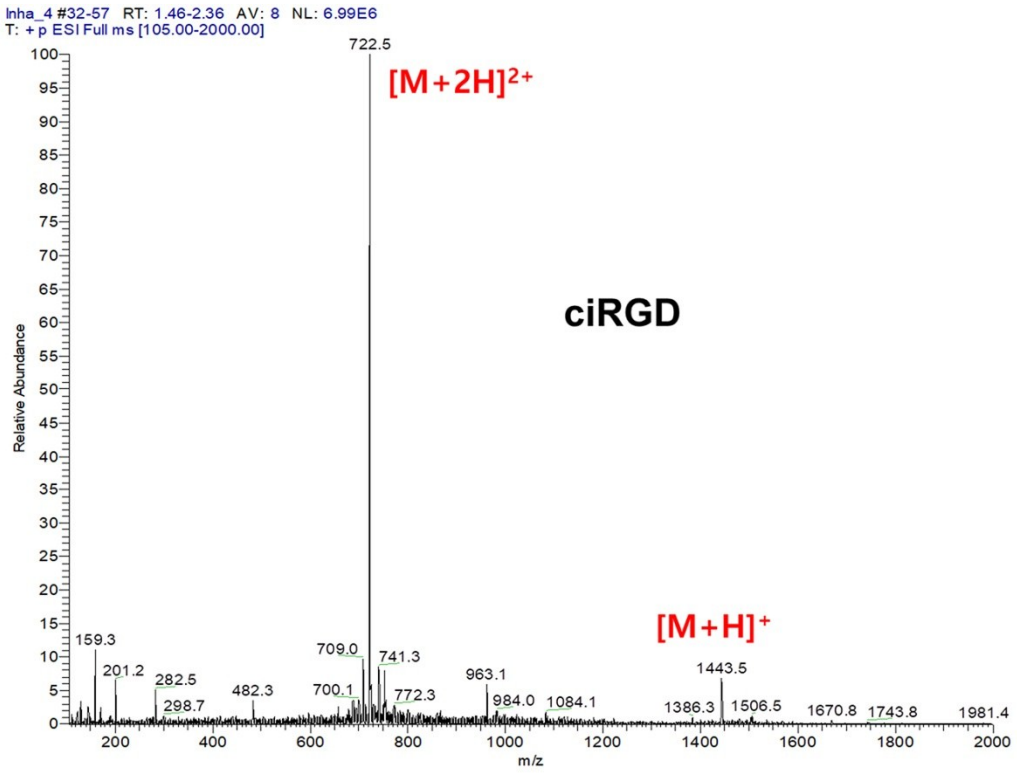

Fig. S4. ESI-Mass spectrum of ciRGD.

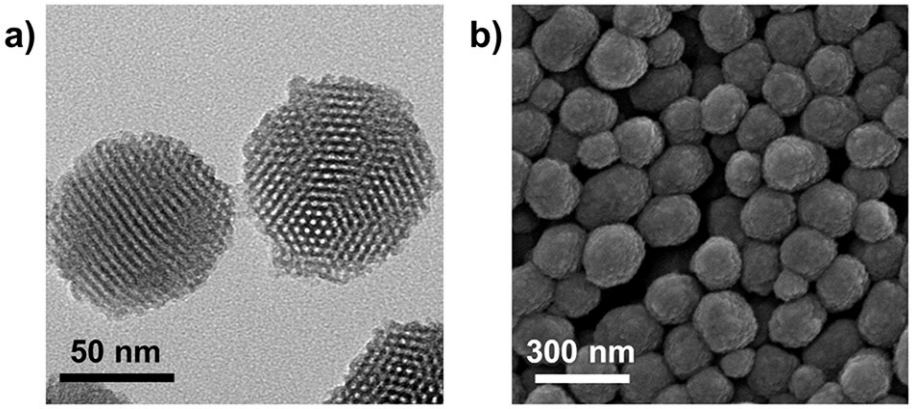

Fig. S5. TEM (a) and FE-SEM (b) images of MCM-41.

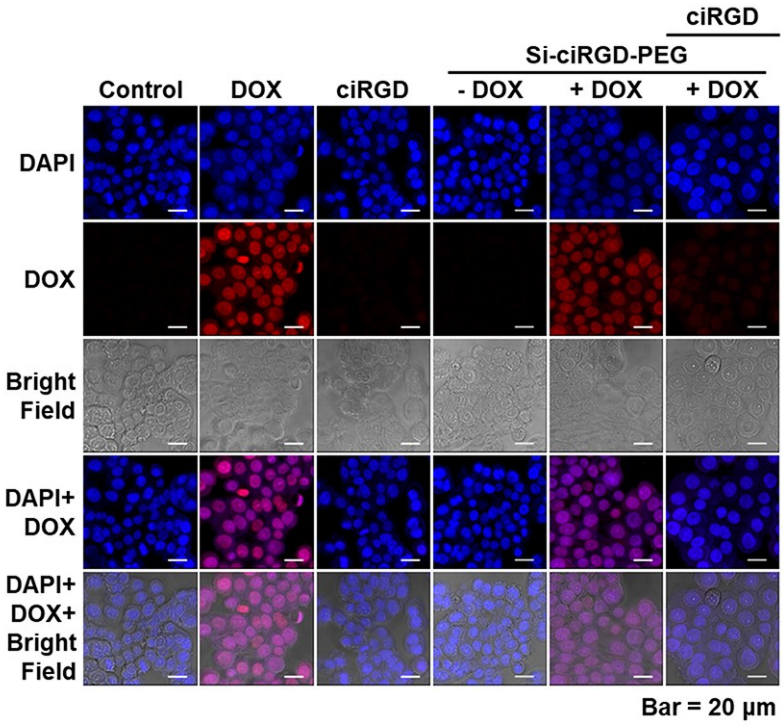

Fig. S6. The assessment of accumulation of DOX released from PEG-WKCRGDRGPDC-SS-Si in the nucleus. Cells were treated with 3 μM DOX or PEG-WKCRGDRGPDC-SS-Si loaded with or without 3 μM DOX in the absence or presence of WKCRGDRGPDC for 12 h. The cells were fixed with 4% PFA, washed three times with PBS and stained with DAPI. The fluorescence intensity of DOX and DAPI-stained nucleus were examined by laser scanning TE2000E confocal microscope (Nikon, tokyo, Japan).

References

[1] W. L. F. Armarego, D. D. Perrin, *Purification of Laboratory Chemicals*, 4th ed., Butterworth-Heinemann, Oxford, U.K, **1996**.

[2] J. Lee, H. Kim, S. Kim, H. Lee, J. Kim, N. Kim, H. J. Park, E. K. Choi, J. S. Lee, C. Kim, *J. Mater. Chem.* **2012**, *22*, 14061-14067.

[3] G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* **1990**, *35*, 161-214.