Electronic Supplementary Material

Cucurbit[7]uril-conjugated dyes as live cell imaging probes: investigation on their cellular uptake and excretion pathways

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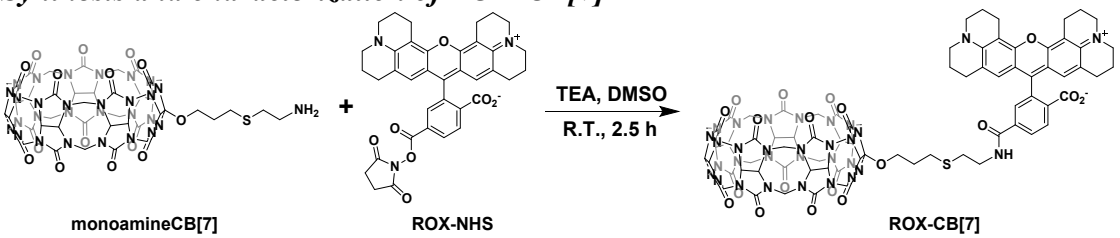
Experimental design

General remarks

Cy3-NHS (Cat. 41020) and ROX-NHS (Cat.42220) are purchased from Lumiprobe Corporation and NHS groups are deactivated by glycine (100 eq.) in PBS for 2 h before utilized as control molecules in cell experiments. Lyso-tracker Green DND-26 (Cat. L7526) was purchased from Molecular Probes (Invitrogen). Endocytosis inhibitors chlorpromazine hydrochloride (C2481) and Genistein (G6649) were purchased from TCI and Sigma-aldrich, respectively. Exocytosis inhibitors Nocodazole (M1404) and Exo1 (E8280) were purchased from Sigma-aldrich. ^{1}H-NMR was recorded on a Bruker Ascend 850 MHz spectrometer. High resolution mass spectrum (HR-ESI-MS) was recorded on a ThemoFisher Q Exactive Plus LC-MS/MS. Human breast carcinoma MCF-7 cell line was obtained from American Type Culture Collection (ATCC) and maintained in high glucose Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 mg/mL streptomycin at 37℃ in a humidified 5% CO_2 incubator. Fluorescence images of cells were obtained on a LSM700 confocal laser scanning microscopes (Carl Zeiss) or a high content analysis system Operetta CLS (Perkin Elmer). The absorbance for MTT analysis was performed on a microplate reader SpectraMax i3 (Molecular Devices) at an absorption wavelength of 490 nm.

Synthesis and characterization of ROX-CB[7]



To a solution of monoamineCB[7] (2.5 mg, 1.9 μmol)[S1] in dimethyl sulfoxide (DMSO, 1 mL), triethylamine (TEA, 50 μL) was added and the reaction mixture was stirred at room temperature under nitrogen atmosphere for 2.5 h. NHS activated ROX (ROX-NHS, 1 mg, 1.58 μmol) was added and stirred for another 2.5 h. After completion of the reaction, the crude product was purified by HPLC to obtain ROX-CB[7] (1.6 mg, 55%). ^{1}H NMR (850 MHz, DMSO-d6) δ (ppm) 8.90 (s, 1H), 8.28 (d, 1H), 8.19 (d, 1H), 7.75 (s, 1H), 6.57 (m, 2H), 5.65 (br m, 14H), 5.41 (br m, 13H), 4.21 (br m, 14H), 3.54 (m, 4H), 3.48 (m, 4H), 3.01 (t, 2H), 2.68 (t, 2H), 2.64 (t, 2H), 2.58 (t, 2H), 2.42 (m, 4H), 1.90 (m, 4H), 1.76 (m, 4H), 1.24 (m, 2H), 1.11 (m, 4H). HR-ESI-MS (*m/z*): $[M+H]^+$ calcd. for $[C_{80}H_{82}N_{31}O_{19}S]^+$, 1812.6119; found, 1812.6137.

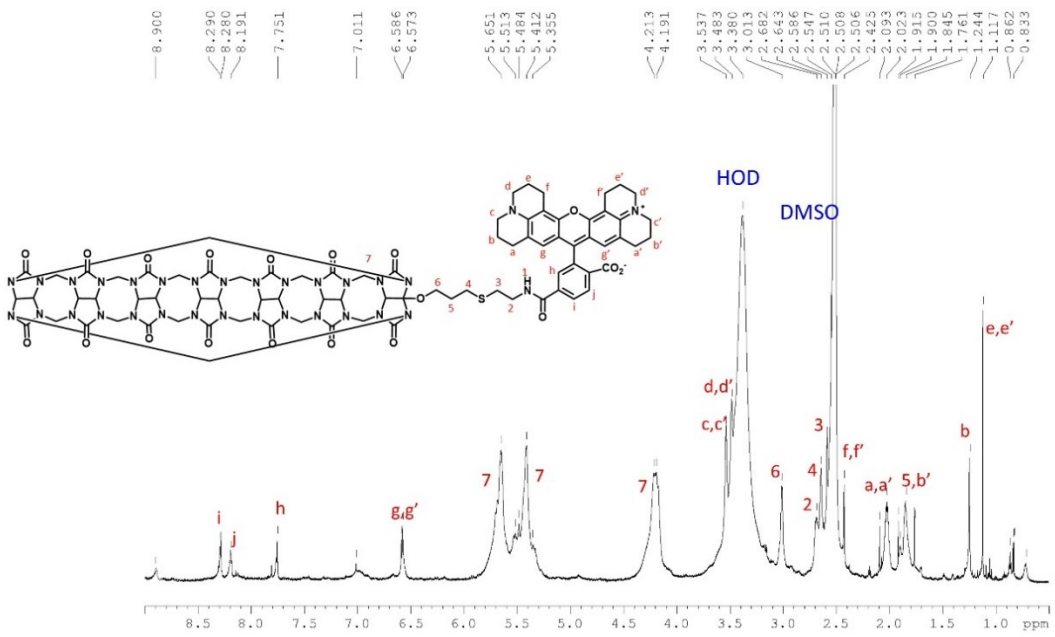


Figure S1. ^{1}H-NMR of ROX-CB[7] in DMSO-d_6.

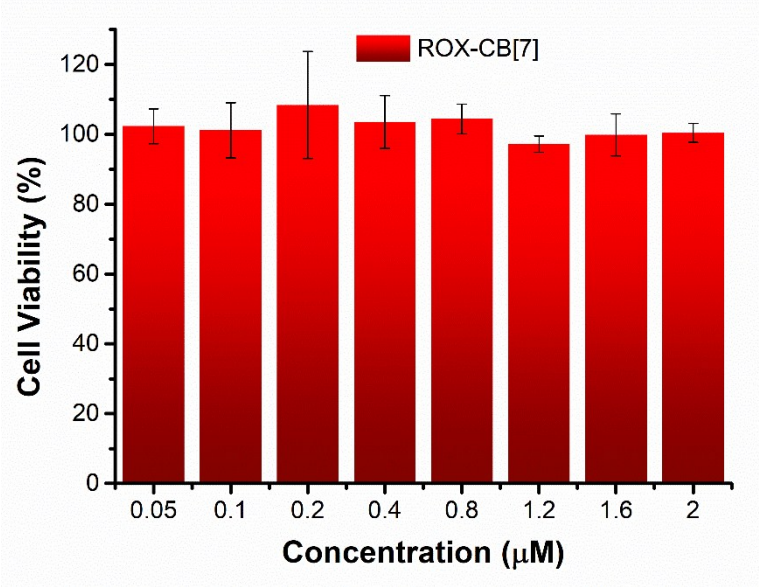


Figure S2. Cytotoxicity of ROX-CB[7] for 24 h to MCF-7 cells.

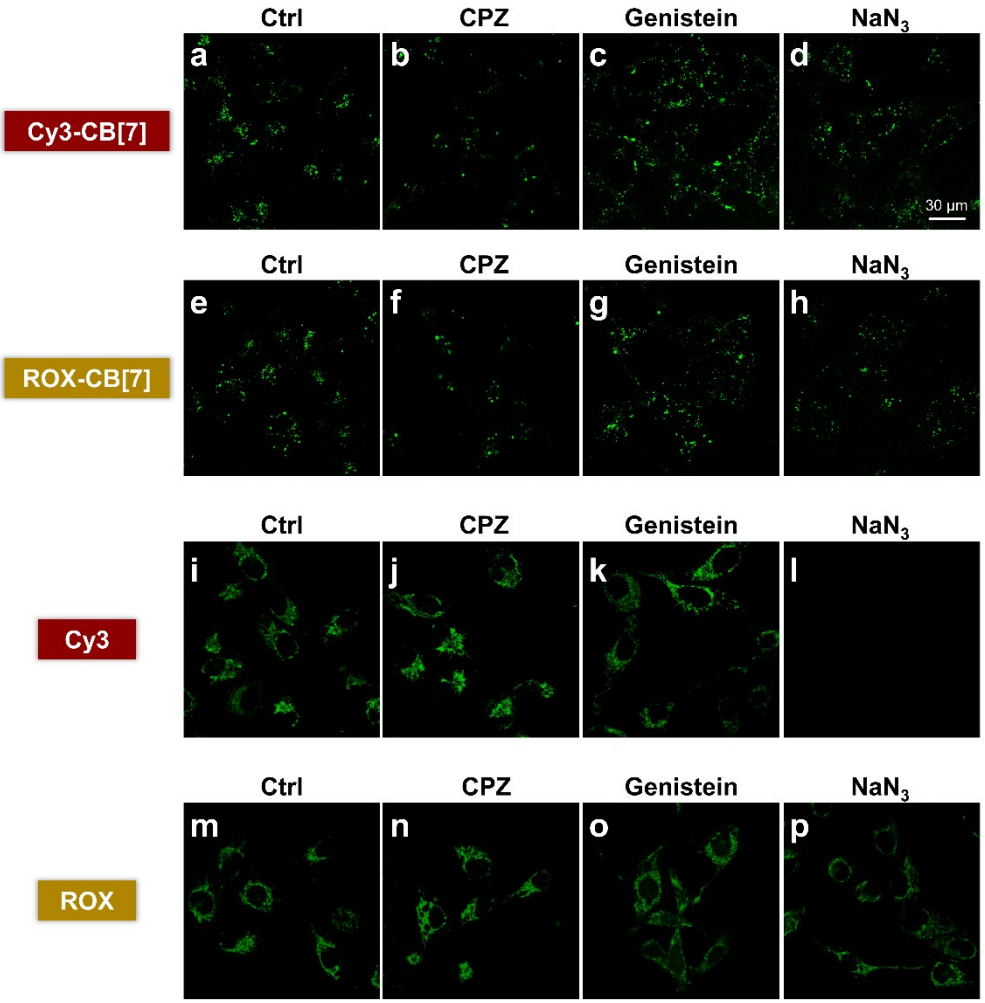


Figure S3. CLSM images of uptake mechanism investigation for Cy3-CB[7] (a~d), ROX-CB[7] (e~h), Cy3 (i~l), and ROX (m~p), respectively. Incubation time is 4 h.

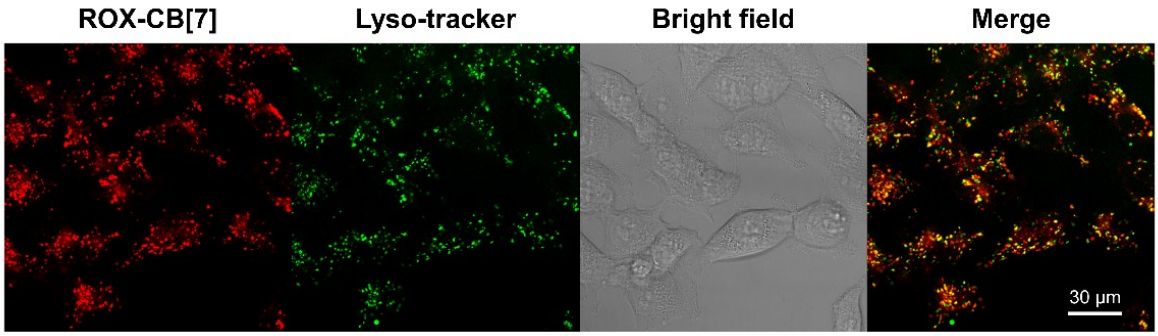


Figure S4. Colocalization of ROX-CB[7] with lyso-tracker.

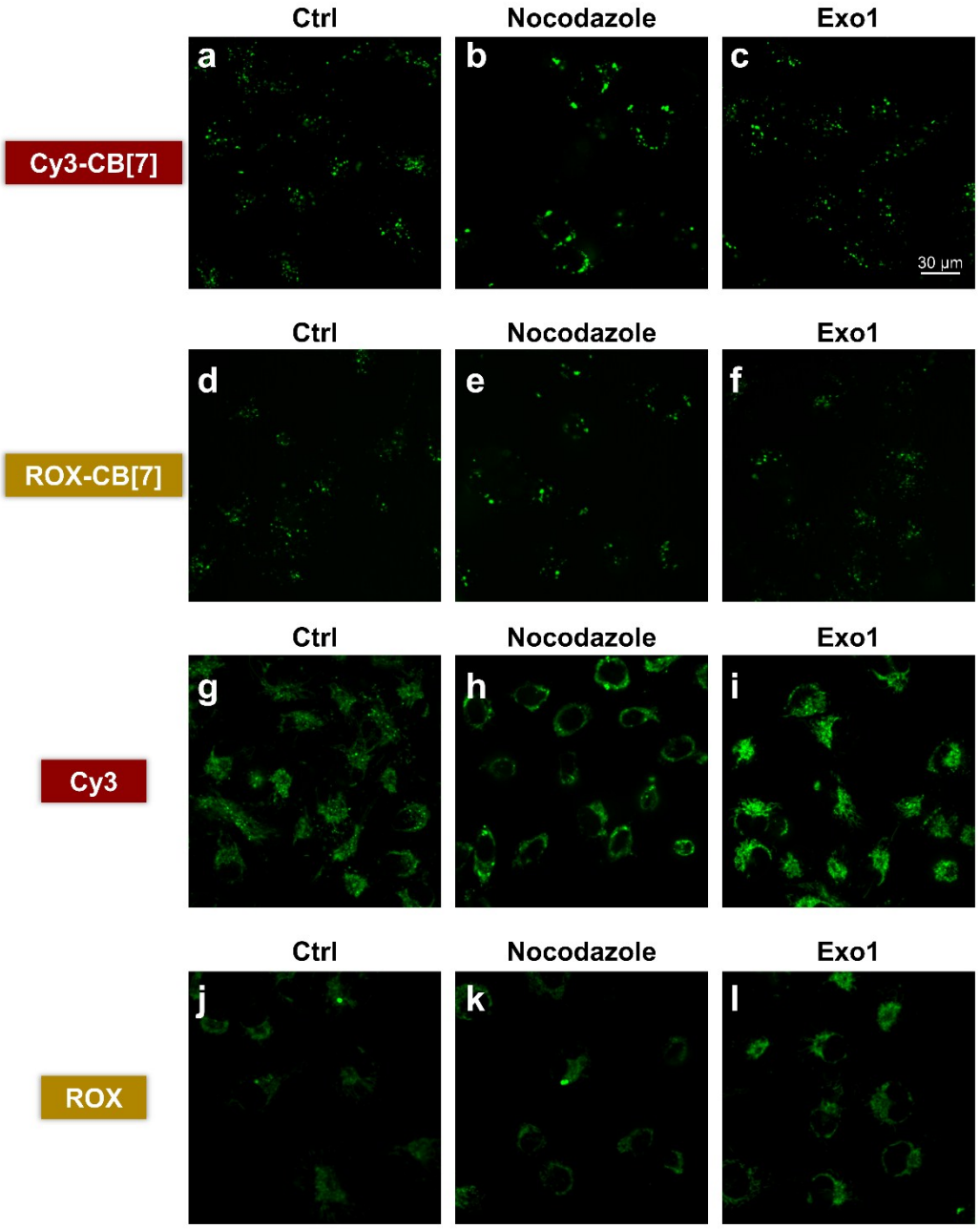


Figure S5. CLSM images for exocytosis mechanism investigation of Cy3-CB[7] (a~c), ROX-CB[7] (d~f), Cy3 (g~i), and ROX (j~l), respectively. The exocytosis period for Cy3-CB[7] and ROX-CB[7] is 12 h. The exocytosis period for Cy3 and ROX is 6 h.

Reference:

S1. M. Li, A. Lee, K. L. Kim, J. Murray, A. Shrinidhi, G. Sung, K. M. Park, K. Kim *Angew. Chem. Int. Ed.* 2018, **57**, 2120-2125.