

## **Electronic Supplementary Information**

### **Supramolecular glyco-poly-cyclodextrin functionalized thin-layer manganese dioxide for targeted stimulus-responsive bioimaging**

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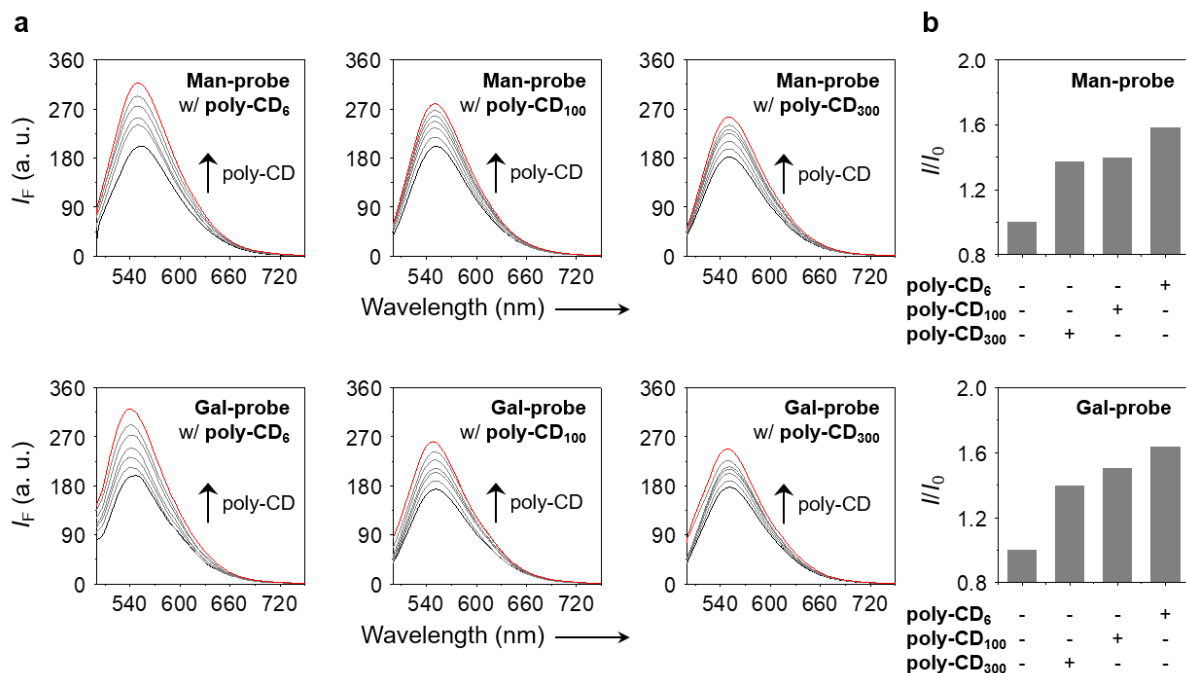
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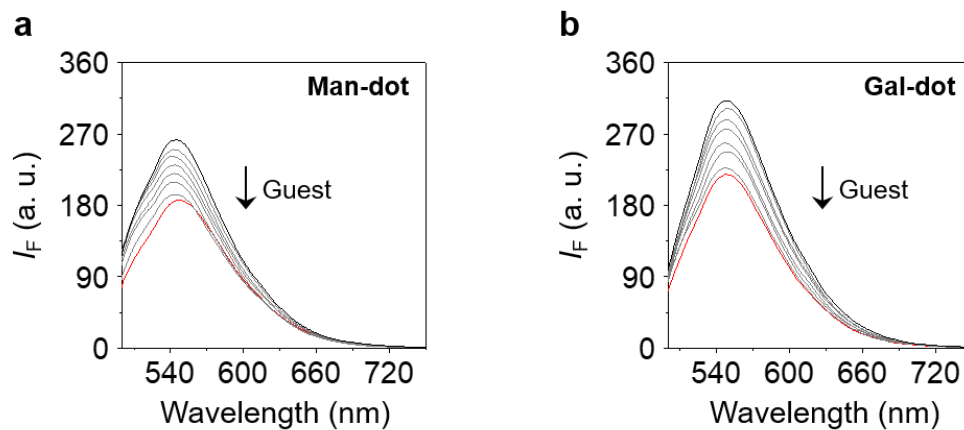
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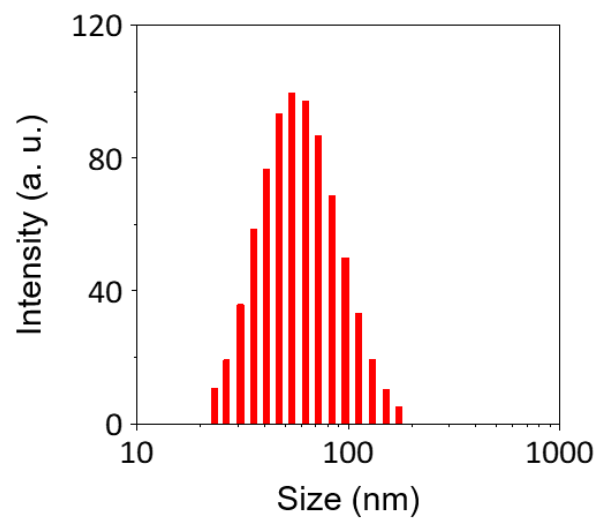
## S1. Additional figures



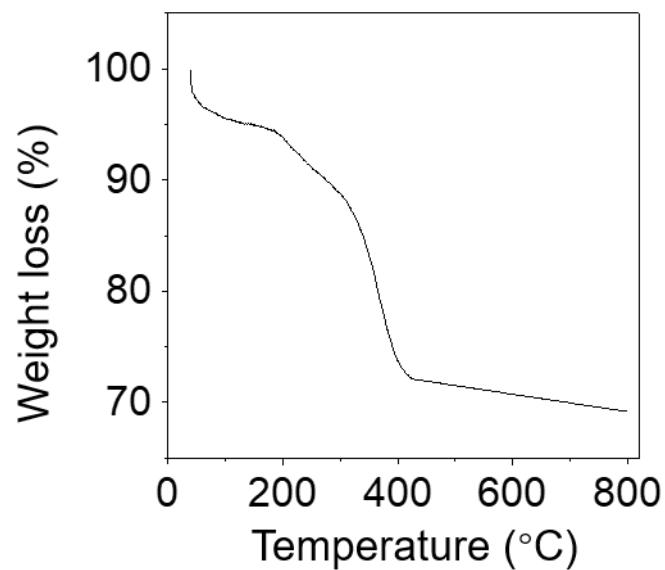
**Figure S1.** (a) Fluorescence spectra of **Man-probe** or **Gal-probe** (1  $\mu\text{M}$ ) with increasing poly-CDs (0-90  $\mu\text{g mL}^{-1}$ ; interval: 15  $\mu\text{g mL}^{-1}$ ). (b) Fluorescence enhancement of **Man-probe** or **Gal-probe** (1  $\mu\text{M}$ ) in the absence and presence of different poly-CDs (80  $\mu\text{g mL}^{-1}$ ), where  $I$  and  $I_0$  are the fluorescence emission intensity (at 550 nm) in the presence and absence of a poly-CD, respectively. All fluorescence spectra were obtained in phosphate buffered saline (PBS, 0.01 M, pH 7.4) with excitation at 450 nm (slit widths ex = 5 nm and em = 5 nm).



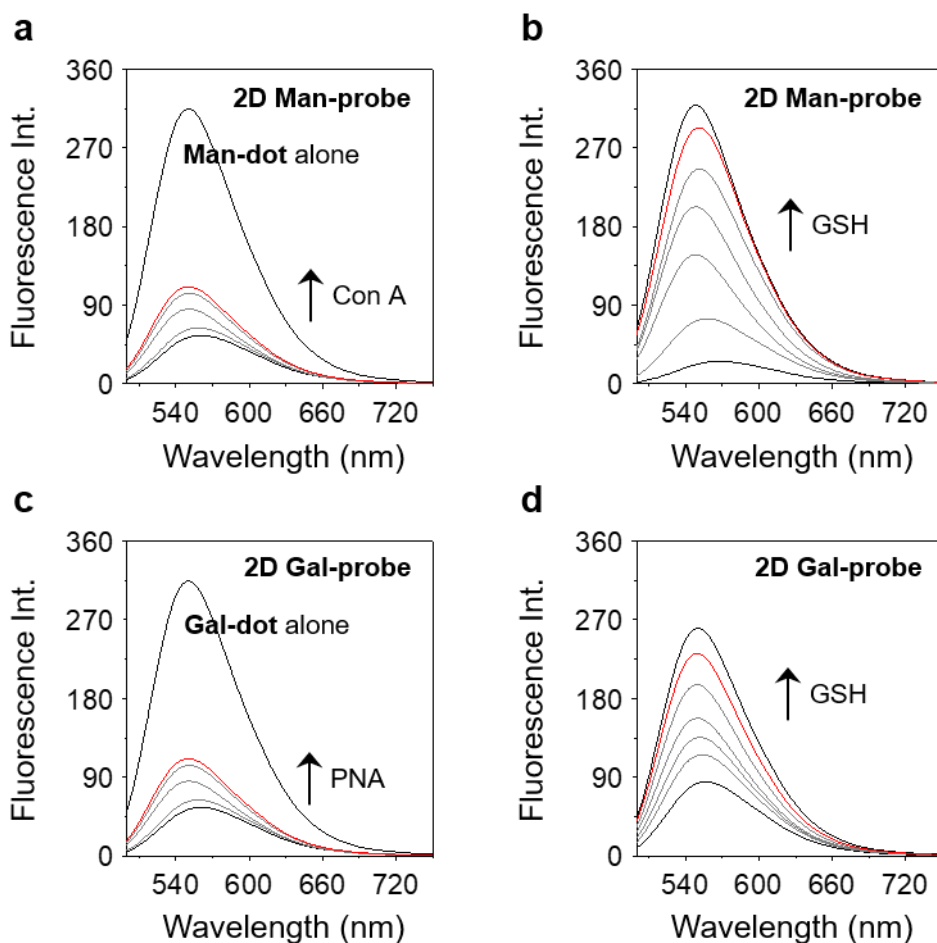
**Figure S2.** Fluorescence spectra of (a) **Man-dot** ( $\text{Man-probe/poly-CD}_{100} = 1 \mu\text{M}/80 \mu\text{g mL}^{-1}$ ) and (b) **Gal-dot** ( $\text{Gal-probe/poly-CD}_6 = 1 \mu\text{M}/80 \mu\text{g mL}^{-1}$ ) with increasing 1-bromonaphthalene (Guest) (0-2 mM; interval: 0.285 mM). All fluorescence spectra were obtained in phosphate buffered saline (PBS, 0.01 M, pH 7.4) with excitation at 450 nm (slit widths  $\text{ex} = 5 \text{ nm}$  and  $\text{em} = 5 \text{ nm}$ ).



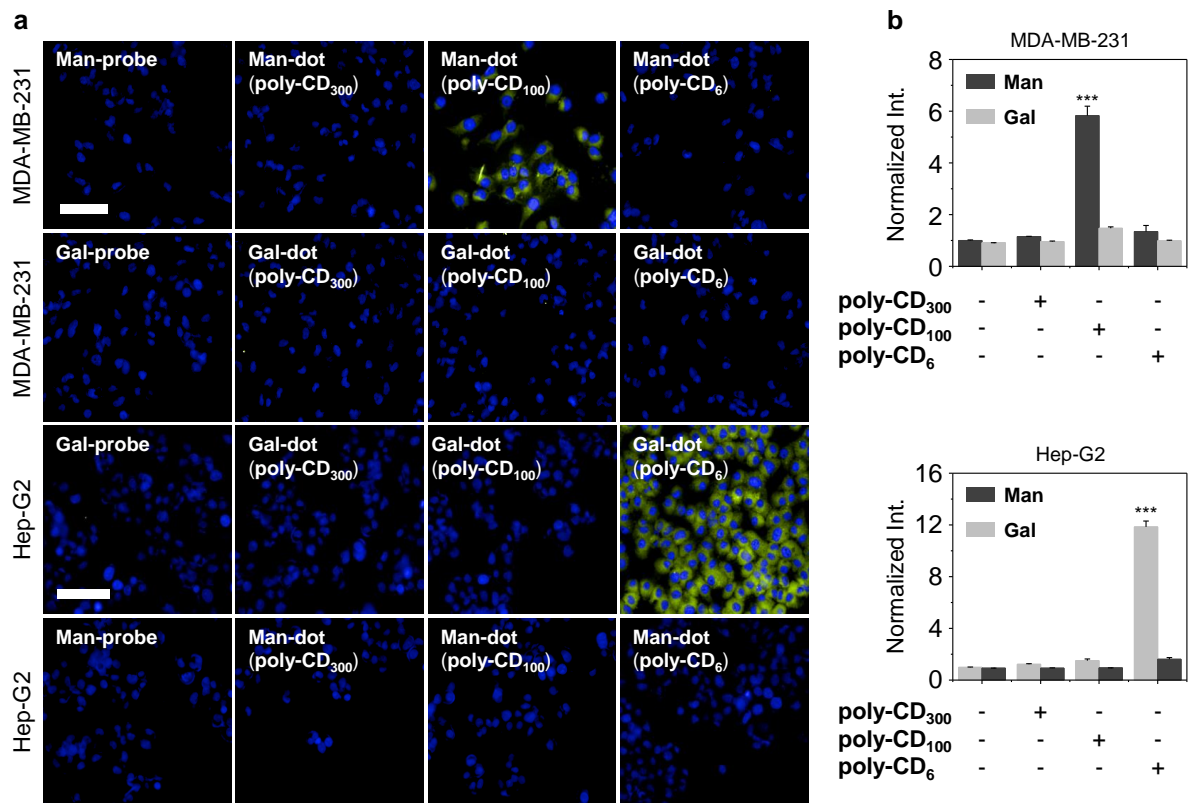
**Figure S3.** Dynamic light scattering of thin-layer MnO<sub>2</sub> (40 μg mL<sup>-1</sup>) measured on Nicomp 380 ZLS.



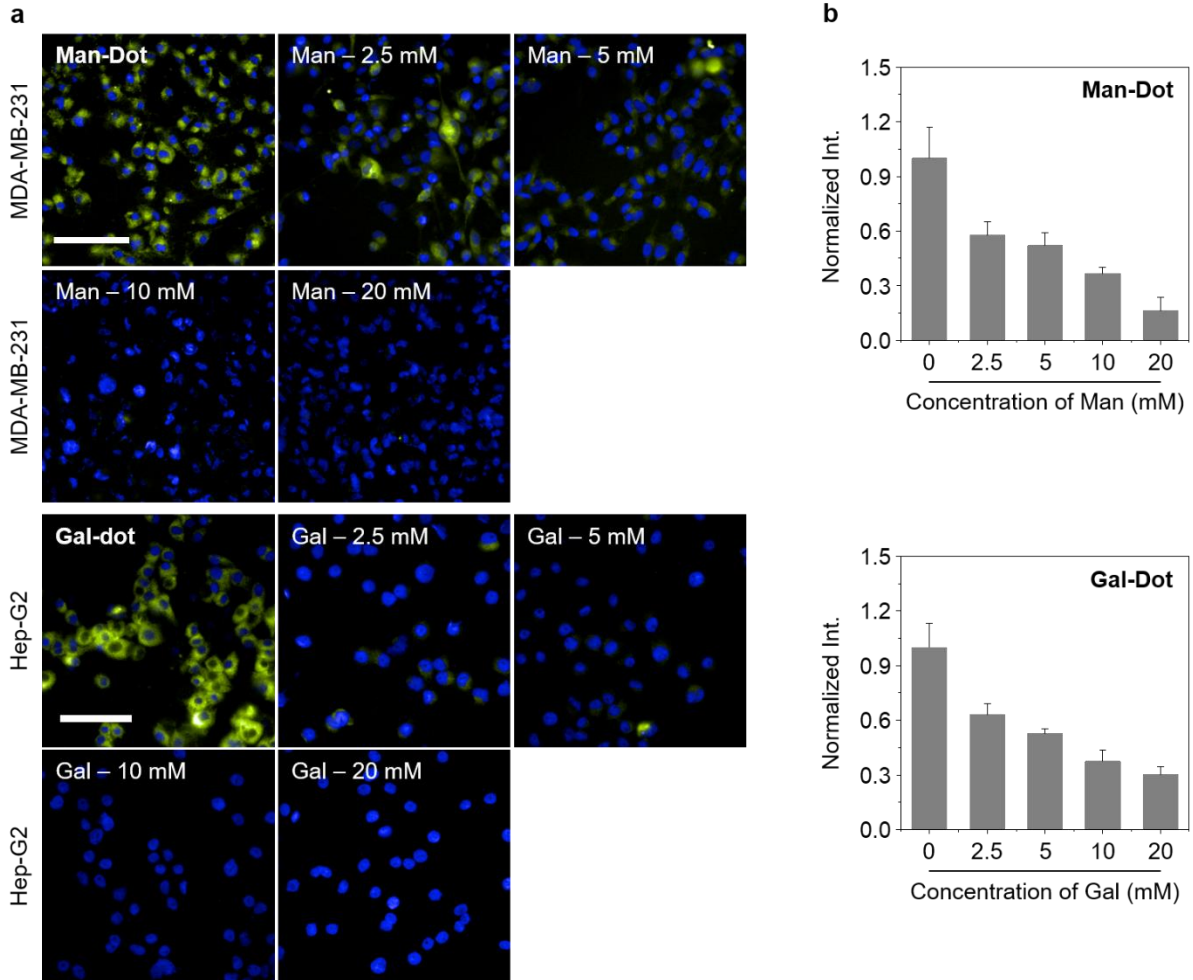
**Figure S4.** Thermogravimetric analysis of **2D Man-probe (Man-probe/poly-CD<sub>100</sub>/2D MnO<sub>2</sub> = 5 μg/500 μg/1500 μg)** measured on PerkinElmer Pyris Diamond TG/DTA. The thermostability of the hybrid material is similar to that of previously developed 2D MnO<sub>2</sub> based hybrid materials (*Electrochem. Commun.*, 2011, **13**, 698).



**Figure S5.** Fluorescence spectra of **2D Man-probe** (**Man-probe/poly-CD<sub>100</sub>/2D MnO<sub>2</sub> = 1  $\mu$ M/80  $\mu$ g mL<sup>-1</sup>/5  $\mu$ g mL<sup>-1</sup>)** with increasing (a) concanavalin A (Con A, a mannose selective lectin; 0-450  $\mu$ M) and (b) GSH (0-450  $\mu$ M). Fluorescence spectra of **2D Gal-probe** (**Gal-probe/poly-CD<sub>6</sub>/2D MnO<sub>2</sub> = 1  $\mu$ M/80  $\mu$ g mL<sup>-1</sup>/5  $\mu$ g mL<sup>-1</sup>)** with increasing (c) peanut agglutinin (PNA, a galactose selective lectin; 0-450  $\mu$ M) and (d) GSH (0-450  $\mu$ M). All fluorescence spectra were obtained in Tris-HCl (0.01 M, pH 7.4) with excitation at 450 nm (slit widths ex = 5 nm and em = 5 nm).

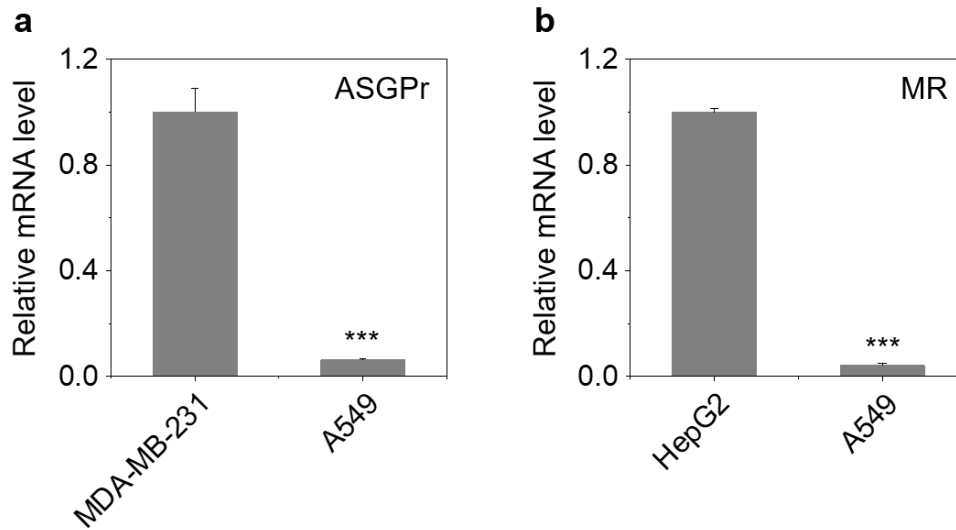


**Figure S6.** Fluorescence (a) imaging and (b) quantification (\*\*\*)  $P < 0.001$  with respect to the glycoprobes alone) of MDA-MB-231 (human triple-negative breast cancer) and Hep-G2 (human liver cancer) cells after treatment of glycoprobes (8  $\mu\text{M}$ ) in the absence or presence of poly-CDs (640  $\mu\text{g mL}^{-1}$ ). Scale bars = 100  $\mu\text{m}$ . The excitation and emission channels used are 460-490 nm and 530-590 nm, respectively. The cell nuclei were stained by Hoechst 33342.



**Figure S7.** Fluorescence (a) imaging and (b) quantification of MDA-MB-231 (human triple-negative breast cancer) and Hep-G2 (human liver cancer) cells with or without pretreatment of free competing monosaccharide (D-mannose (Man) and D-galactose (Gal) for **Man-dot** and **Gal-dot**, respectively) upon incubation with glycodots (glycoprobe/poly-CD = 1  $\mu\text{M}$ /80  $\mu\text{g mL}^{-1}$ ). The excitation and emission channels used are 460-490 nm and 530-590 nm, respectively. The cell nuclei were stained by Hoechst 33342.





**Figure S8.** Relative mRNA level of (a) ASGPr (asialoglycoprotein receptor) determined in MDA-MB-231 and A549 cells and (b) MR (mannose receptor) determined in Hep-G2 and A549 cells by real-time quantitative polymerase chain reaction (\*\*\*) $P < 0.001$ ).

## S2. Experimental section

**General.** All purchased chemicals and reagents are of analytical grade. Proteins were purchased from Sigma-Aldrich.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AM 400 MHz spectrometer. High resolution mass spectra (HRMS) were recorded with a Waters Micromass LCT mass spectrometer. High Performance Liquid Chromatography (HPLC) was measured on a Shimadzu Prominence Series equipment. Ultrapure water was obtained from a Milli-Q Integral Pure/Ultrapure Water Production unit.

**Preparation of Man-dot and Gal-dot.** To prepare the **Man-dot** (**Man-probe/poly-CD<sub>100</sub>** = 1  $\mu\text{M}/80 \mu\text{g mL}^{-1}$ ) and **Gal-dot** (**Gal-probe/poly-CD<sub>6</sub>** = 1  $\mu\text{M}/80 \mu\text{g mL}^{-1}$ ), a solvent displacement method was carried out with water and dimethylsulfoxide (DMSO). A DMSO solution of **Man-probe** or **Gal-probe** (10  $\mu\text{M}$ ) and an aqueous solution of poly-CDs were mixed and stirred vigorously. The glycoprobe molecules with an adamantine tail could be inserted to the hydrophobic cavity of CD due to hydrophobic “host-guest” interactions. The mixture was then centrifuged at 8000 rpm for 20 min to remove residual glycoprobe molecules. Then, the solution of **Man-dot** and **Gal-dot** was obtained from dialysis.

**Cell culture.** Hep-G2 cells were cultured in Dulbecco’s Modified Eagle’s Medium (Gibco, Gland Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA), and A549 cells were cultured in F-12 medium (Gibco, Gland Island, NY, USA) supplemented with 10% FBS in a humidified atmosphere of 5%  $\text{CO}_2$  and 95% air at 37 °C and split when the cells reached 85% confluency. MDA-MB-231 cells were maintained in RPMI-1640 medium containing 5% FBS at 37°C with 5%  $\text{CO}_2$  and 95% air and split when the cells reached 90% confluency.

**Cell imaging.** Cells (Hep-G2 and MDA-MB-231 – 25000/well; A549 – 15000/well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. For direct fluorescent quantitation, the cells were incubated with **Man-dot** (**Man-probe/poly-CD<sub>100</sub>** = 8  $\mu\text{M}/640 \mu\text{g mL}^{-1}$ ) and **Gal-dot** (**Gal-probe/poly-CD<sub>6</sub>** = 8  $\mu\text{M}/640 \mu\text{g mL}^{-1}$ ) for 15 min. For competition assay, the Hep-G2 and MDA-MB-231 cells were pre-incubated with free D-galactose and D-mannose for 1 h, followed by incubation with **Man-dot** and **Gal-dot** for 15 min, respectively. For GSH-responsive fluorescence imaging, cells were pre-treated with NEM (Sigma, E3876) of 500  $\mu\text{M}$  for 30 min to reduce GSH concentration in living cells. Then, both cell lines were washed three times with cell culture

medium. To enhance the GSH concentration, cells were treated with GSH (Sigma, G4251) of 300  $\mu\text{M}$  for 30 min. Then, the cells were incubated with 2D glycoprobes (glycoprobe/poly-CD/2D  $\text{MnO}_2 = 4 \mu\text{M}/320 \mu\text{g mL}^{-1}/12 \mu\text{g mL}^{-1}$ ) for 15 min. The cell nuclei were stained with Hoechst 33342 ( $5 \mu\text{g mL}^{-1}$ ) at  $37^\circ\text{C}$  in a humidified atmosphere of 5%  $\text{CO}_2$  in air for 5 min, and gently washed with PBS three times before imaging. The fluorescence images were recorded using an Operetta high content imaging system and quantified by the Columbus image data analysis system (Perkinelmer, US).

**Real-time quantitative PCR.** Total RNA was isolated from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript<sup>®</sup> RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup>. Real-time PCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, CA, USA). GAPDH was detected as the housekeeping gene. Primers for qPCR were as follows:

GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'

and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'

ASGPR1 forward, 5'-CTGGACAATGAGGAGAGTGAC-3'

and reverse, 5'-TTGAAGCCCGTCTCGTAGTC-3'

Mannose Receptor forward, 5'-GCAGCTCTGGGAACTGGAT-3'

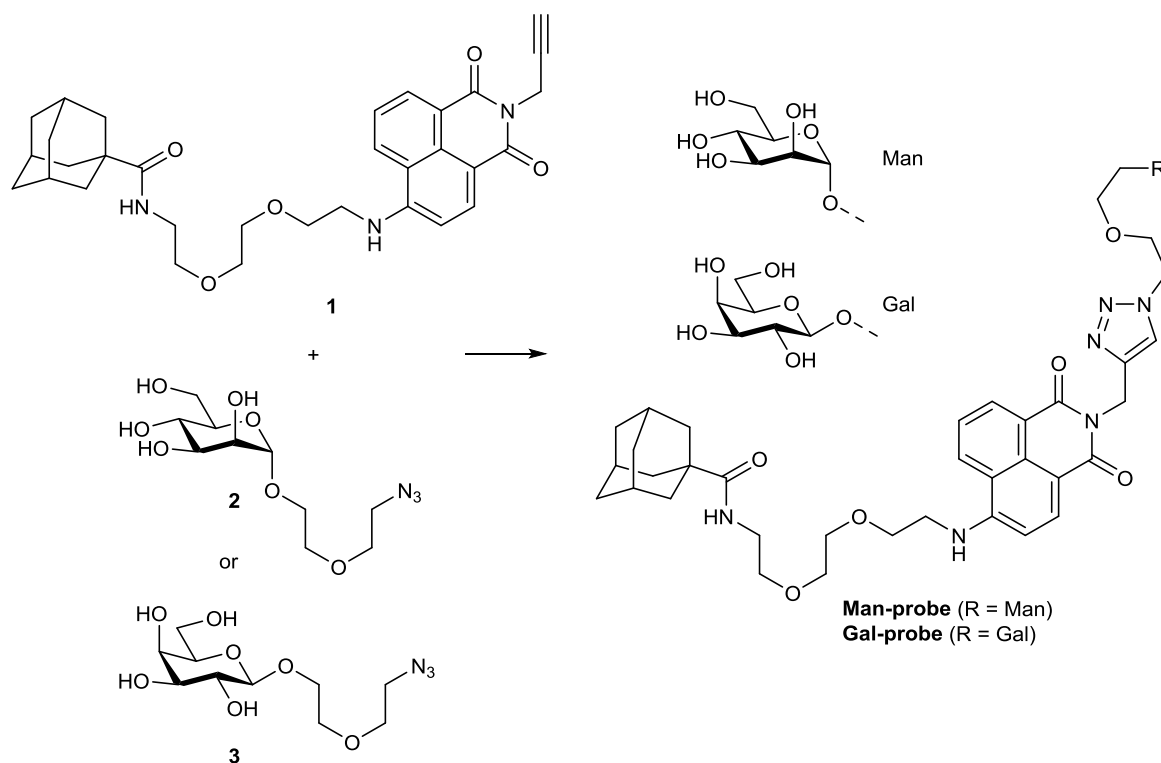
and reverse, 5'-TTGCCTGGTGTCCAGTAGGA-3'

**Synthesis of Man-probe (Scheme S1) (compounds 1 and 2 were prepared according to previous protocols).**<sup>1,2</sup> From **1** (200 mg, 0.68 mmol) and **2** (380 mg, 0.68 mmol), column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 9:1$ , v/v) afforded **Man-probe** as a yellow solid (526 mg, 91%);  $R_f$  0.68 ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 5:1$ , v/v).

<sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  8.30 (t,  $J = 8.0$  Hz, 2H), 8.12 (d,  $J = 8.0$  Hz, 1H), 8.02 (s, 1H), 7.45 (dd,  $J_1 = 16.0$  Hz,  $J_2 = 8.0$  Hz, 1H), 6.58 (d,  $J = 8.0$  Hz, 1H), 5.36 (s, 2H), 4.76 (s, 1H), 4.56 (t,  $J = 4.0$  Hz, 2H), 3.87-3.81 (m, 6H), 3.76-3.70 (m, 5H), 3.72-3.66 (m, 3H), 3.59-3.53 (m, 8H), 3.36-3.33 (d,  $J = 8.0$  Hz, 2H), 1.83 (s, 5H), 1.73-1.68 (m, 2H), 1.65-1.57 (m, 7H), 1.48-1.40 (m, 3H). <sup>13</sup>C NMR (101 MHz, MeOD)  $\delta$  173.9, 165.9, 165.1, 152.5, 135.9, 132.4, 132.4, 132.3, 131.0, 130.0, 126.1, 125.4, 123.0, 121.6, 109.3, 105.2, 101.7, 74.7, 72.6, 72.1, 71.6, 71.3, 71.2, 70.8, 70.0, 68.7, 67.6, 66.7, 62.9, 51.9, 51.5, 43.7, 40.2, 37.9, 33.7, 30.1, 23.8.

HRMS (ESI,  $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{43}\text{H}_{58}\text{N}_6\text{O}_{12}^+$  851.4191, found 851.4178.

HPLC:  $t_R$  6.473 min of methanol ( $0.6 \text{ mL min}^{-1}$ ), purity 95.9%.

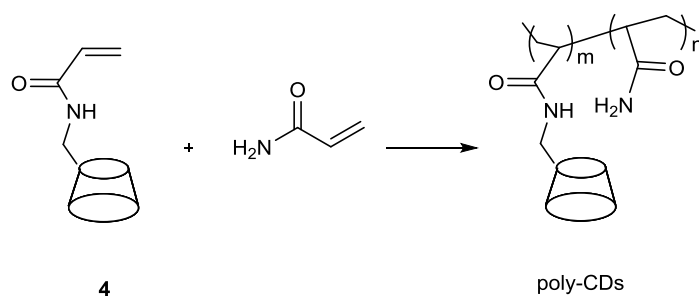


**Scheme S1.** Reagents and conditions:  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and Sodium ascorbate in  $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}/t\text{-BuOH}$  (2:1:1, v/v/v) at  $60^\circ\text{C}$ .

**Synthesis of Gal-probe (Scheme S1) (Gal-probe were prepared according to previous protocols).<sup>2</sup>** From **1** (200 mg, 0.68 mmol) and **2** (380 mg, 0.68 mmol), column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 9:1$ , v/v) afforded **Gal-probe** as a yellow solid (526 mg, 91%);  $R_f$  0.68 ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 5:1$ , v/v).

HRMS (ESI,  $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{43}\text{H}_{58}\text{N}_6\text{O}_{12}^+$  851.4191, found 851.4166.

HPLC:  $t_R$  6.949 min of methanol ( $0.6 \text{ mL min}^{-1}$ ), purity 96%.



**Scheme S2.** Reagents and conditions: KPS in  $\text{DMSO}/\text{H}_2\text{O}$  (1/1, v/v) at  $60^\circ\text{C}$ .

**General procedure for the preparation of poly-CDs.** From **4** (1 eq) and acrylamide (6, 100 or 300 eq), a radical polymerization was carried out with potassium persulfate (KPS) (0.002 g, 0.008 mmol, 0.2 eq) as radical initiator at 60 °C under an argon atmosphere in DMSO/H<sub>2</sub>O (1/1, v/v) for 12 h. The resulting mixture was added into methanol, and the precipitate was repeatedly washed with methanol to give purified polymers. The copolymerization ratio was calculated from the integration ratio of <sup>1</sup>H NMR signals. The calculated ratio was mainly in accordance with the initial amounts of the CD monomer.

**Synthesis of poly-CD<sub>6</sub> (Scheme S2).** From **4** (0.05 g, 0.042 mmol, 1 eq) and acrylamide (0.018 g, 0.25 mmol, 6 eq), the radical polymerization afforded poly-CD<sub>6</sub> (0.04 g, yield 64%) as a white powder. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, 25 °C): δ 4.98 (s, 7H), 3.87-3.78 (m, 24H), 3.57-3.49 (m, 18H), 2.31-2.11 (broad, principal chain protons), 1.73-1.42 (broad, principal chain protons). GPC (H<sub>2</sub>O): Mn (PDI) = 25.1 kDa (1.10).

**Synthesis of poly-CD<sub>100</sub> (Scheme S2).** From **4** (0.05 g, 0.042 mmol, 1 eq) and acrylamide (0.3 g, 4.2 mmol, 100 eq), the radical polymerization afforded poly-CD<sub>100</sub> (0.23 g, yield 65%) as a white powder. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, 25 °C): δ 2.24-2.09 (broad, principal chain protons), 1.68-1.56 (broad, principal chain protons). GPC (H<sub>2</sub>O): Mn (PDI) = 18 kDa (3.65).

**Synthesis of poly-CD<sub>300</sub> (Scheme S2).** From **4** (0.05 g, 0.042 mmol, 1 eq) and acrylamide (0.9 g, 12.63 mmol, 300 eq), the radical polymerization afforded poly-CD<sub>300</sub> (0.55 g, yield 58%) as a white powder. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, 25 °C): δ 2.57-1.93 (broad, principal chain protons), 1.66-1.53 (broad, principal chain protons). GPC (H<sub>2</sub>O): Mn (PDI) = 47 kDa (4.42).

**Table S1** – Characterization of poly-CDs.<sup>[a]</sup>

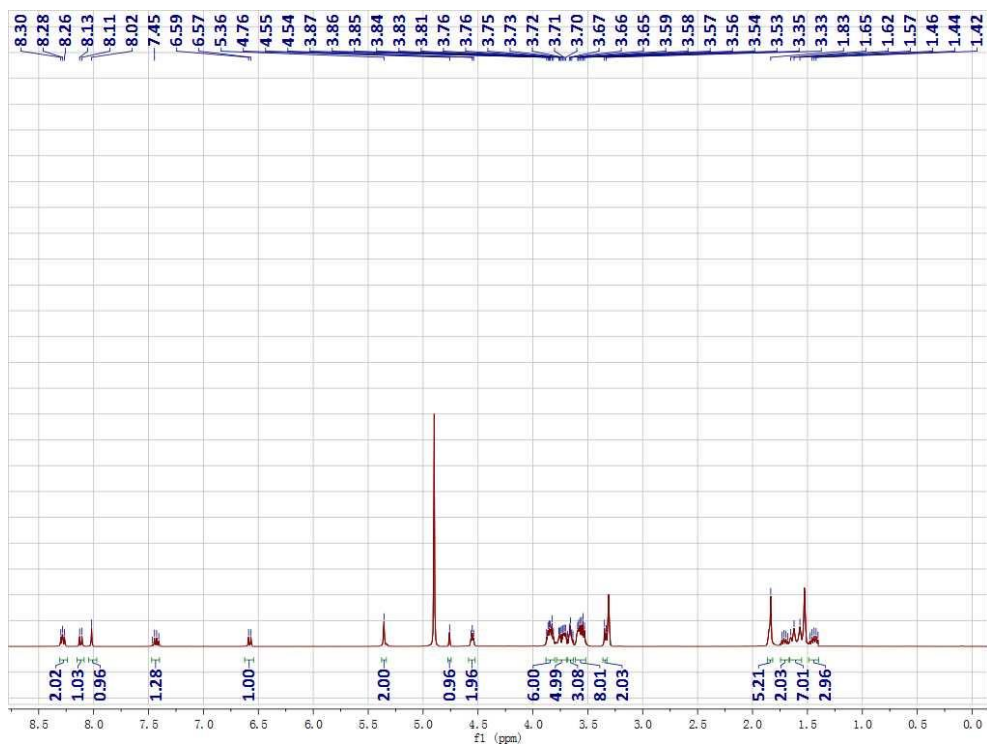
Sample	Mn × 10 <sup>3</sup>	Polydispersity	Theoretical composition (mono-CD:acrylamide)	Experimental composition (mono-CD:acrylamide) <sup>[b]</sup>
poly-CD <sub>6</sub>	25.1	1.10	1:6	1:6
poly-CD <sub>100</sub>	18.0	3.65	1:100	n. a. <sup>[c]</sup>
poly-CD <sub>300</sub>	47.0	4.42	1:300	n. a.

[a] Mn and Polydispersity (PDI) were determined by aqueous GPC.

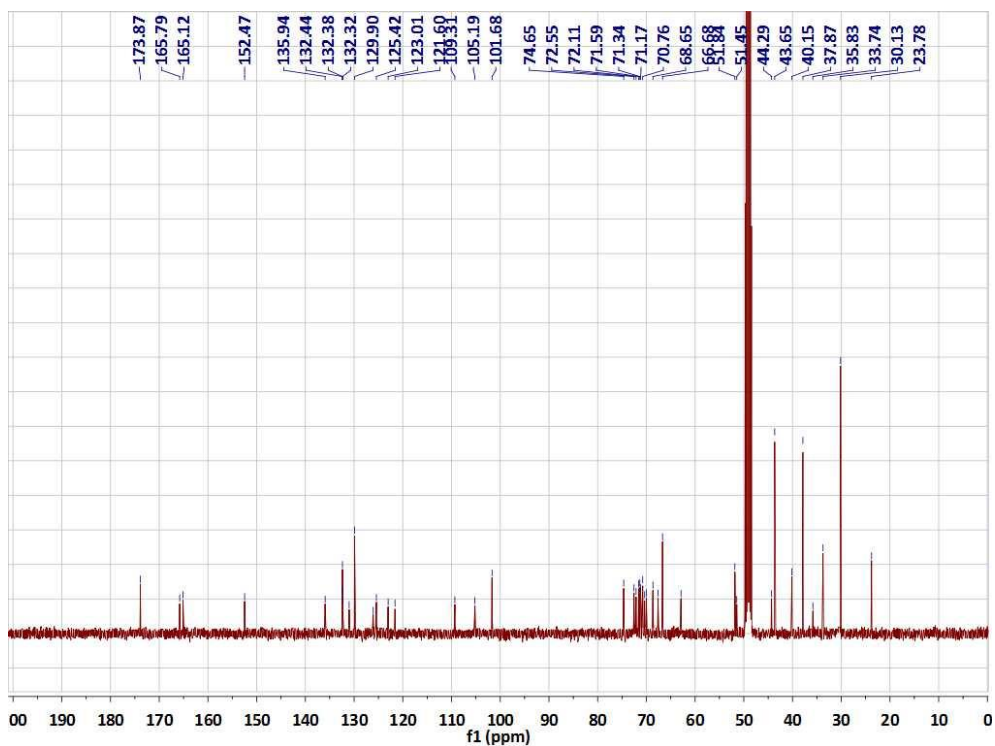
[b] The copolymerization ratio was calculated from the integration ratio of <sup>1</sup>H NMR signals.

[c] n. a. = Not Available.

### S3. Spectral copies of new compounds



<sup>1</sup>H NMR of Man-probe



<sup>13</sup>C NMR of Man-probe

#### **S4. Additional references**

1. Z. Li, L. Sun, Y. Zhang, A. P. Dove, R. K. O'Reilly and G. Chen, *ACS Macro Lett.*, 2016, **5**, 1059-1064.
2. X. L. Hu, Y. Zang, J. Li, G. R. Chen, T. D. James, X. P. He and H. Tian, *Chem. Sci.*, 2016, **7**, 4004-4008.
3. H. Chen, X. Ma, S. Wu and H. Tian, *Angew. Chem. Int. Ed.*, 2014, **53**, 14149-14152.