

## Insulin-Based Regulation of Glucose-functionalized Nanoparticle Uptake in Muscle Cells

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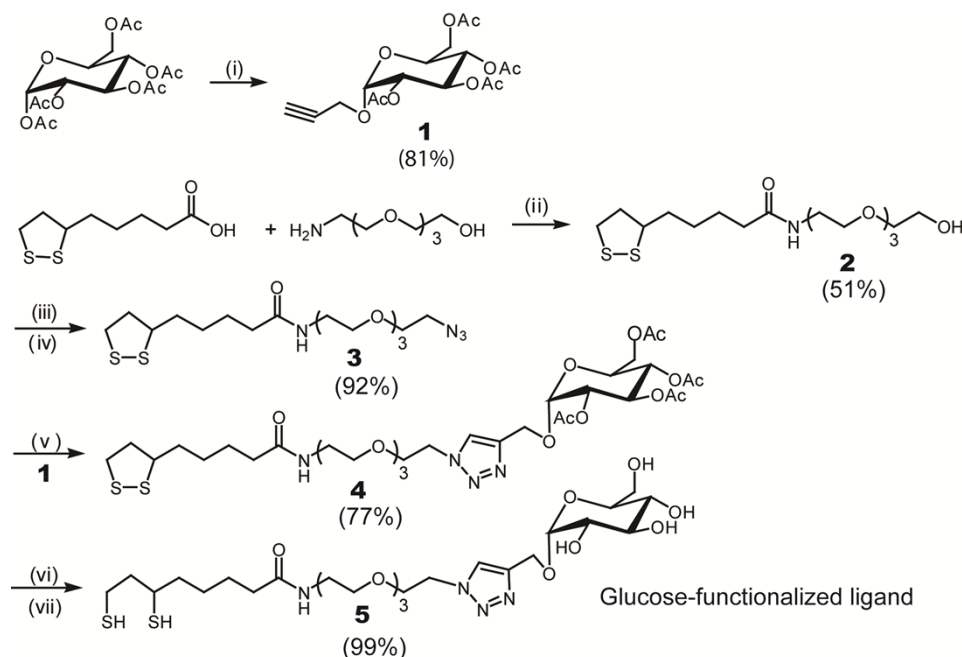
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### Supporting information

#### Synthesis of glucose-functionalized ligand



**Scheme S1.** Synthetic route of the glucose-functionalized ligand. Reagents and conditions: (i)  $\text{SnCl}_4$ , 2-propynol, DCM, r.t., 24 h; (ii) EDC, HOBT, DIPEA, DCM, r.t., 24 h; (iii)  $\text{MsCl}$ ,  $\text{NEt}_3$ , DCM, 0 °C to r.t., 24 h; (iv)  $\text{NaN}_3$ , DMF/EtOH, 85 °C, 24 h; (v)  $\text{CuSO}_4$ , NaAsc,  $\text{H}_2\text{O}/\text{DCM}$ , r.t., 24 h; (vi) NaOMe, dry MeOH, r.t., 5 min. (vii)  $\text{NaBH}_4$ , EtOH/  $\text{H}_2\text{O}$ , r.t., 1 h.

**Compound 1:** A solution of  $\beta$ -D-glucose pentaacetate (1 mmol) in 20 ml of dry DCM was treated with  $\text{SnCl}_4$  (1 mmol) for 10 min before the addition of 2-propynol (1.5 mmol). After 4 h at room temperature, the reaction was quenched with saturated  $\text{NaHCO}_3$  solution. The product was purified by chromatography column with EtOAc/Hexane as eluent (1:1, v:v) in 81% yield.

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): 5.23 (t, 1H, H-3), 5.09 (t, 1H, H-4), 5.00 (t, 1H, H-2), 4.75 (d, 1H, H-1), 4.35 (d, 2H, OCH<sub>2</sub>CCH), 4.27 (m, 1H, H-6), 4.14 (dd, 1H, H-6'), 3.73 (m, 1H, H-5), 2.47 (t, 1H, OCH<sub>2</sub>CCH), 2.08 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.00 (s, 3H, OAc) ppm.

**Compound 2:** Thioctic acid (2.56 g, 12.4 mmol) was dissolved in 75 ml dry DCM and cooled to 0 °C for 5 min. EDC (2.78 g, 14.5 mmol), HOBt (1.9 g, 12.4 mmol) and DIPEA (1.603 g, 12.4 mmol) were added to the solution, and the solution was purged with N<sub>2</sub> gas for several min. Amino terminated tetraethylene glycol (2 g, 10.3 mmol) was added to the reaction mixture and stirred at r.t. for 24 h. Then, the reaction mixture was diluted with DCM, filtered with Celite, and washed with brine and water. Compound **2** was obtained by using column chromatography with 100 % EtOAc as eluent in 51% yield.

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) major peaks assigned: 3.73 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>OH), 3.71-3.51 (m, 12H, TEG), δ 3.46 (t, 2H, CONHCH<sub>2</sub>CH<sub>2</sub>), 3.21-3.08 (brm, 2H), 2.51-2.42 (brm, 1H), 2.19 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.76-1.62 (m, 4H), 1.52-1.40 (m, 2H) ppm.

**Compound 3:** Compound **2** was dissolved in 50 ml of dry THF, and cooled to 0 °C in ice bath. NEt<sub>3</sub> and CH<sub>3</sub>SO<sub>2</sub>Cl was added to the solution under stirring condition at 0 °C. The reaction was stirred overnight at r.t. Then, the reaction mixture was diluted with THF and washed with 5 % HCl, NaHCO<sub>3</sub> and water. After removing THF, the reaction mixture was redissolved in 20 ml of DMF/EtOH (30:70, v:v), and NaN<sub>3</sub> was added at 80 °C. Finally, the mixture was heated to 90 °C for overnight. Compound **3** was obtained by using column chromatography with MeOH/EtOAc (1:10, v:v) as eluent in 92% yield.

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) major peaks assigned: 3.68 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>OH), 3.65-3.42 (m, 12H, TEG), 3.40 (t, 2H, CONHCH<sub>2</sub>CH<sub>2</sub>), 3.21-3.08 (brm, 2H), 2.51-2.42 (brm, 1H), 2.19 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.76-1.61 (m, 4H), 1.52-1.41 (m, 2H) ppm.

**Compound 4:** A solution of **3** (300 mg, 0.74 mmol) and **1** (313 mg, 0.81 mmol) in 5 ml of DCM was added to another solution of CuSO<sub>4</sub> (17.7 mg, 0.11 mmol) and sodium ascorbate (66 mg, 0.33 mmol) in 5 ml of water. The reaction mixture was stirred at r.t. for 24 h. The aqueous and organic phases were separated when the stirring was stopped. The aqueous layer was washed with DCM (3 × 10 mL) and the organic layer was dried with NaSO<sub>4</sub>. The product was purified by chromatography column with MeOH/EtOAc (1:10, v:v) as eluent in 77% yield.

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): 7.73 (s, 1H, CCHN), 5.19 (t, 1H, H-3), 5.10 (t, 1H, H-4), 5.00 (t, 1H, H-2), 4.92 (d, 1H, OCH<sub>2</sub>CNCH<sub>2</sub>), 4.83 (d, 1H, OCH<sub>2</sub>CNCH<sub>2</sub>), 4.70 (d, 1H, H-1), 4.59-4.52 (m, 2H, NCH<sub>2</sub>CH<sub>2</sub>O), 4.27 (dd, 1H, H-6), 4.13 (dd, 1H, H-6'), 3.89 (t, 2H, NCH<sub>2</sub>CH<sub>2</sub>O), 3.78 - 3.52 (m, 12H, TEG), 3.45 (t, 2H, CONHCH<sub>2</sub>CH<sub>2</sub>), 3.22-3.08 (brm, 2H), 2.51-2.42 (brm, 1H), 2.19 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.74-1.61 (m, 4H), 1.53-1.39 (m, 2H) ppm.

**Compound 5:** NaOMe (123 µl, 0.63 mmol) was added to a solution of **4** (500 mg, 0.63 mmol) in dry and deoxygenated MeOH (5 ml). After 5 min, the reaction mixture was neutralized with <sup>+</sup>H Dowex resin, filtered with Millex-GP syringe filter (0.22 µm), and the solvent was evaporated. The mixture (0.5 g) was dissolved in 10 ml of EtOH / water (1:4) with stirring, and NaBH<sub>4</sub> (0.075 g) was added to the solution. The reaction mixture was stirred for 1 h and became colorless. The reaction mixture was

diluted with water and extracted with  $\text{CHCl}_3$ . The combined organic phase was dried over  $\text{Na}_2\text{SO}_4$ . Compound **5** can be obtained after evaporating the solvent.

$^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ): 8.07 (s, 1H, click), 5.0 (t, 1H, H-3), 4.8 (t, 1H,  $J = 10$  Hz, H-4), 4.58 (m, 2H,  $\text{NCH}_2\text{CH}_2\text{O}$ ), 4.40 (dd, 1H, H-6), 4.13 (dd, 1H, H-6'), 3.89 (t, 2H,  $\text{NCH}_2\text{CH}_2\text{O}$ ), 3.70-3.48 (m, 12H, TEG), 3.31 (t, 2H,  $\text{CONHCH}_2\text{CH}_2$ ), 3.23-3.07 (brm, 2H), 2.51-2.41 (brm, 1H), 2.20 (t, 2H,  $\text{CH}_2\text{CH}_2\text{CONH}$ ), 1.77-1.55 (m, 4H), 1.53-1.36 (m, 2H) ppm.

MS (ESI-MS) calculated for  $\text{C}_{25}\text{H}_{46}\text{N}_4\text{O}_{10}\text{S}_2$  626.27, found 647.59 [M-Na] and 663.60 [M-K]

The synthesis of galactose-functionalized ligand was followed the same procedure as glucose-functionalized ligand while using  $\beta$ -D-galactose pentaacetate as a starting material.

$^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ): 8.11 (s, 1H, click), 4.90 (t, 1H, H-3), 4.77 (t, 1H,  $J = 10$  Hz, H-4), 4.64 (m, 2H,  $\text{NCH}_2\text{CH}_2\text{O}$ ), 4.47 (dd, 1H, H-6), 3.97 (dd, 1H, H-6'), 3.90 (t, 2H,  $\text{NCH}_2\text{CH}_2\text{O}$ ), 3.84-3.50 (m, 12H, TEG), 3.33 (t, 2H,  $\text{CONHCH}_2\text{CH}_2$ ), 3.27-3.11 (brm, 2H), 2.52-2.40 (brm, 1H), 2.23 (t, 2H,  $\text{CH}_2\text{CH}_2\text{CONH}$ ), 1.67-1.51 (m, 4H), 1.46-1.30 (m, 2H) ppm.

MS (ESI-MS) calculated for  $\text{C}_{25}\text{H}_{46}\text{N}_4\text{O}_{10}\text{S}_2$  626.27, found 647.69 [M-Na] and 663.70 [M-K]

Abbreviations:

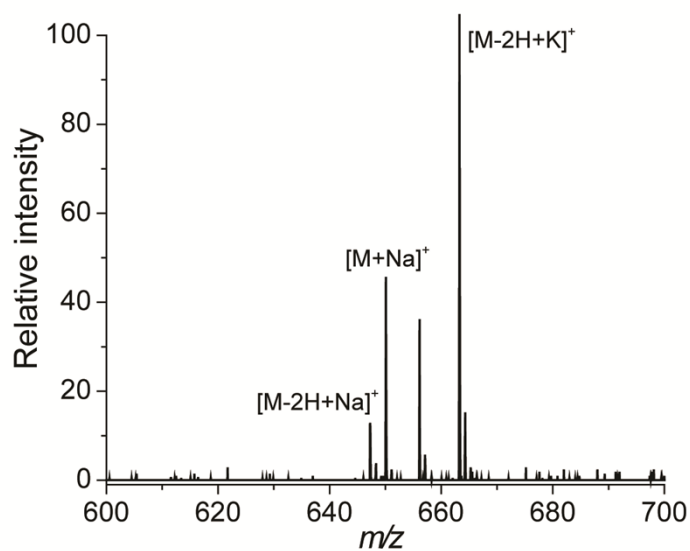
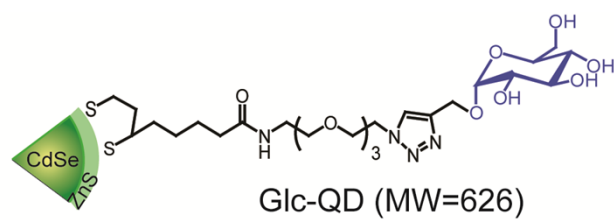
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), hydroxybenzotriazole (HOBt), N,N-diisopropylethylamine (DIPEA), and mesyl chloride (MsCl), dichloromethane (DCM), dimethylformamide (DMF), tetra-ethylene glycol (TEG).

### Electrospray Ionization Mass Spectrometry (ESI-MS)

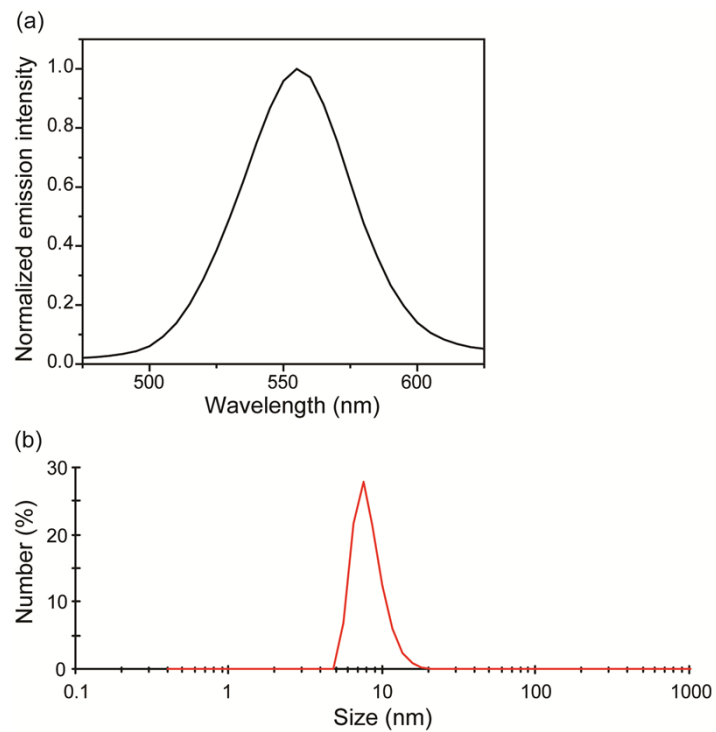
The mass spectra were acquired at positive mode on a Bruker Esquire-LC (Billerica, MA) quadrupole ion trap mass spectrometer, equipped with an electrospray ionization source. The electrospray needle voltage was set to 3.5 kV, and the capillary temperature was kept as 300 °C. Usually a voltage of 30 V was applied to skimmer 1 and a voltage of 80 - 90 V was applied to the capillary offset. Samples ( $\sim 20$   $\mu\text{M}$ ) were delivered at 200  $\mu\text{L}/\text{h}$  using a syringe pump.

### Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)

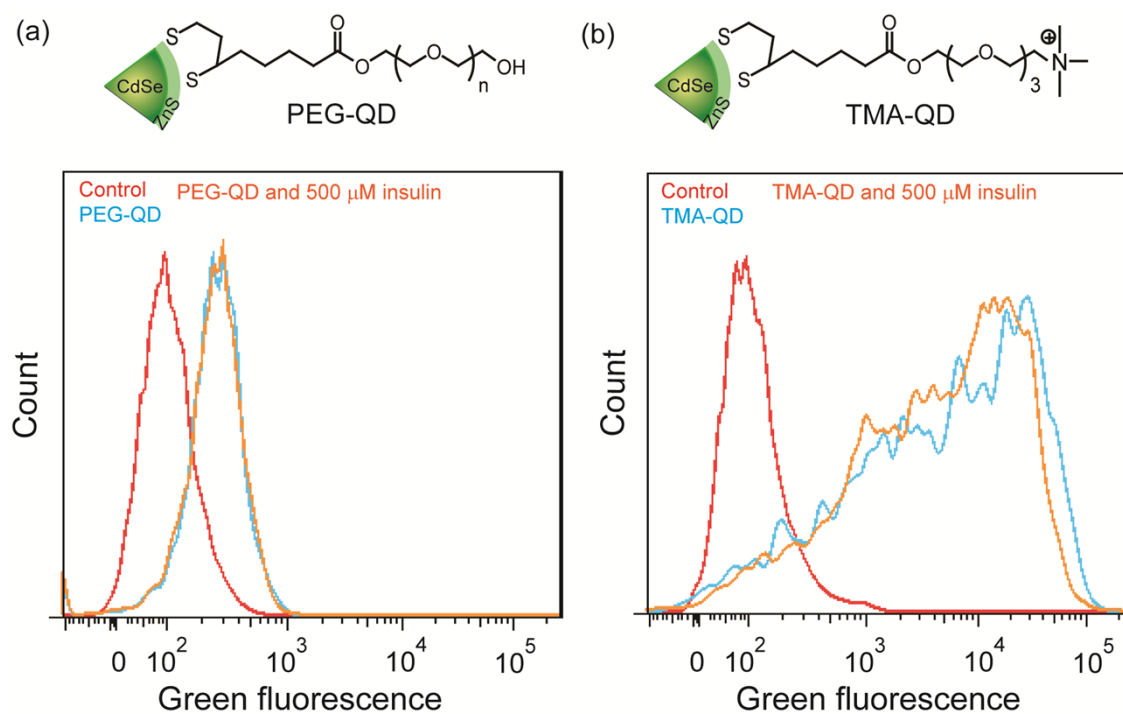
The MALDI-MS analyses were acquired at positive mode on a Bruker Omnisflex time-of-flight mass spectrometer (Omniflex), equipped with a 337 nm nitrogen laser, a 1.0 m flight tube, and a stainless steel sample target. All mass spectra were acquired in reflectron mode. The reflectron voltage was set to 20 kV and the accelerating voltage of 19 kV. On this instrument, an average of 50 laser shots was fired to acquire each spectrum, and a laser power of 10 % was used, which corresponds to approximately 30  $\mu\text{J}/\text{pulse}$ . A saturated  $\alpha$ -CHCA stock solution was prepared in 70 % acetonitrile, 30 %  $\text{H}_2\text{O}$ , and 0.1 % trifluoroacetic acid, and to this stock solution was added an equal volume of a 2  $\mu\text{M}$  solution of Glc-QDs. 1  $\mu\text{L}$  of this mixture was applied to target, and after allowing it to dry, the MALDI-MS analysis was performed.



**Fig. S1** MALDI-MS spectrum of Glc-QD.



**Fig. S2** (a) Emission spectrum and (b) hydrodynamic diameter of Glc-QDs.



**Fig. S3** Two control experiments were performed for the insulin effect on regulating QD uptake. (a) Polyethylene glycol-functionalized QDs (PEG-QDs) and (b) trimethylammonium-functionalized QDs (TMA-QDs) were incubated in differentiated C2C12 cells for 4 h with the treatment of insulin. The concentration of QDs used in these experiments was 250 nM.