

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

Yi-Cheun Yeh[†], Sung Tae Kim[†], Rui Tang, Bo Yan and Vincent M. Rotello*

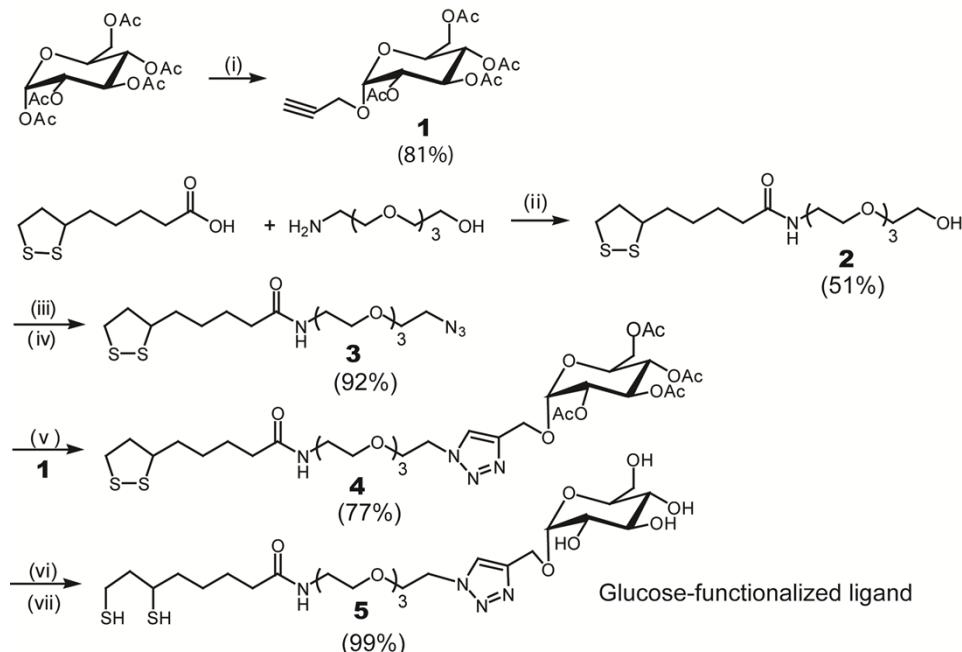
[†]These authors contributed equally to this work.

*Correspondence should be addressed to V. M. R. (rotello@chem.umass.edu)

*Corresponding author. Tel.: (+1) 413-545-2058; Fax: (+1) 413-545-4490; E-mail address: [\(V. M. R.\)](mailto:rotello@chem.umass.edu)

Supporting information

Synthesis of glucose-functionalized ligand



Scheme S1. Synthetic route of the glucose-functionalized ligand. Reagents and conditions: (i) SnCl_4 , 2-propynol, DCM, r.t., 24 h; (ii) EDC, HOBT, DIPEA, DCM, r.t., 24 h; (iii) MsCl , NEt_3 , DCM, 0 °C to r.t., 24 h; (iv) NaN_3 , DMF/EtOH, 85 °C, 24 h; (v) CuSO_4 , NaAsc , $\text{H}_2\text{O}/\text{DCM}$, r.t., 24 h; (vi) NaOMe , dry MeOH , r.t., 5 min. (vii) NaBH_4 , $\text{EtOH}/\text{H}_2\text{O}$, r.t., 1 h.

Compound 1: A solution of β -D-glucose pentaacetate (1 mmol) in 20 ml of dry DCM was treated with 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 NaHCO_3 solution. The product was purified by chromatography column with $\text{EtOAc}/\text{Hexane}$ as eluent (1:1, v:v) in 81% yield.

¹H NMR (400 MHz, CD₃OD): 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, OCH₂CCH), 4.27 (m, 1H, H-6), 4.14 (dd, 1H, H-6'), 3.73 (m, 1H, H-5), 2.47 (t, 1H, OCH₂CCH), 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 N₂ 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.

¹H NMR (400 MHz, CD₃OD) major peaks assigned: 3.73 (t, 2H, CH₂CH₂OH), 3.71-3.51 (m, 12H, TEG), δ 3.46 (t, 2H, CONHCH₂CH₂), 3.21-3.08 (brm, 2H), 2.51-2.42 (brm, 1H), 2.19 (t, 2H, CH₂CH₂CONH), 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. NEt₃ and CH₃SO₂Cl 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, NaHCO₃ and water. After removing THF, the reaction mixture was redissolved in 20 ml of DMF/EtOH (30:70. v:v), and NaN₃ 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.

¹H NMR (400 MHz, CD₃OD) major peaks assigned: 3.68 (t, 2H, CH₂CH₂OH), 3.65-3.42 (m, 12H, TEG), 3.40 (t, 2H, CONHCH₂CH₂), 3.21-3.08 (brm, 2H), 2.51-2.42 (brm, 1H), 2.19 (t, 2H, CH₂CH₂CONH), 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 CuSO₄ (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 NaSO₄. The product was purified by chromatography column with MeOH/EtOAc (1:10, v:v) as eluent in 77% yield.

¹H NMR (400 MHz, CD₃OD): 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, OCH₂CNCH₂), 4.83 (d, 1H, OCH₂CNCH₂), 4.70 (d, 1H, H-1), 4.59-4.52 (m, 2H, NCH₂CH₂O), 4.27 (dd, 1H, H-6), 4.13 (dd, 1H, H-6'), 3.89 (t, 2H, NCH₂CH₂O), 3.78 - 3.52 (m, 12H, TEG), 3.45 (t, 2H, CONHCH₂CH₂), 3.22-3.08 (brm, 2H), 2.51-2.42 (brm, 1H), 2.19 (t, 2H, CH₂CH₂CONH), 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 ¹H 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 NaBH₄ (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 CHCl_3 . The combined organic phase was dried over Na_2SO_4 . Compound **5** can be obtained after evaporating the solvent.

^1H NMR (400 MHz, CD_3OD): 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 β -D-galactose pentaacetate as a starting material.

^1H NMR (400 MHz, CD_3OD): 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 (HOBr), 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 (~20 μM) 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 Omniflex 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 α -CHCA stock solution was prepared in 70 % acetonitrile, 30 % H_2O , and 0.1 % trifluoroacetic acid, and to this stock solution was added an equal volume of a 2 μM solution of Glc-QDs. 1 μL of this mixture was applied to target, and after allowing it to dry, the MALDI-MS analysis was performed.

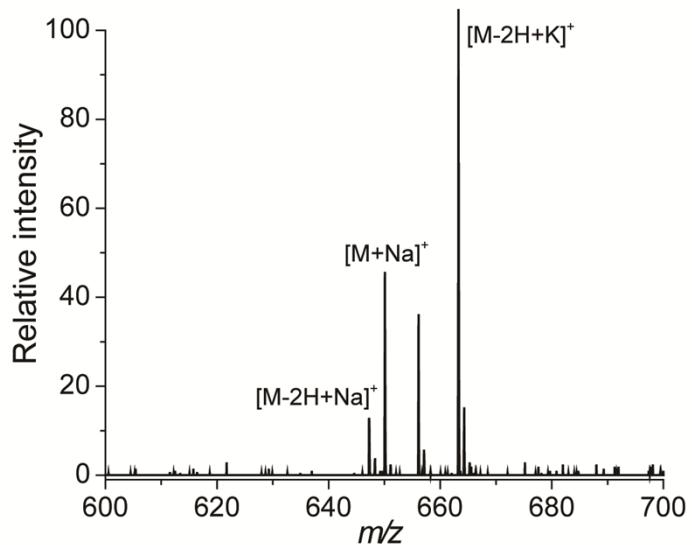
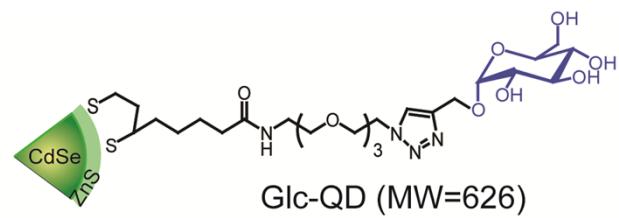


Fig. S1 MALDI-MS spectrum of Glc-QD.

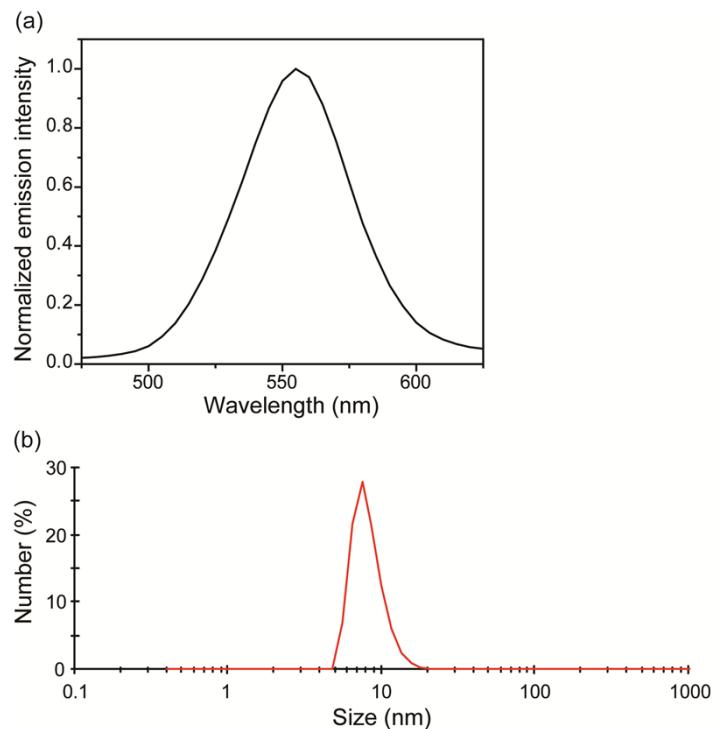


Fig. S2 (a) Emission spectrum and (b) hydrodynamic diameter of Glc-QDs.

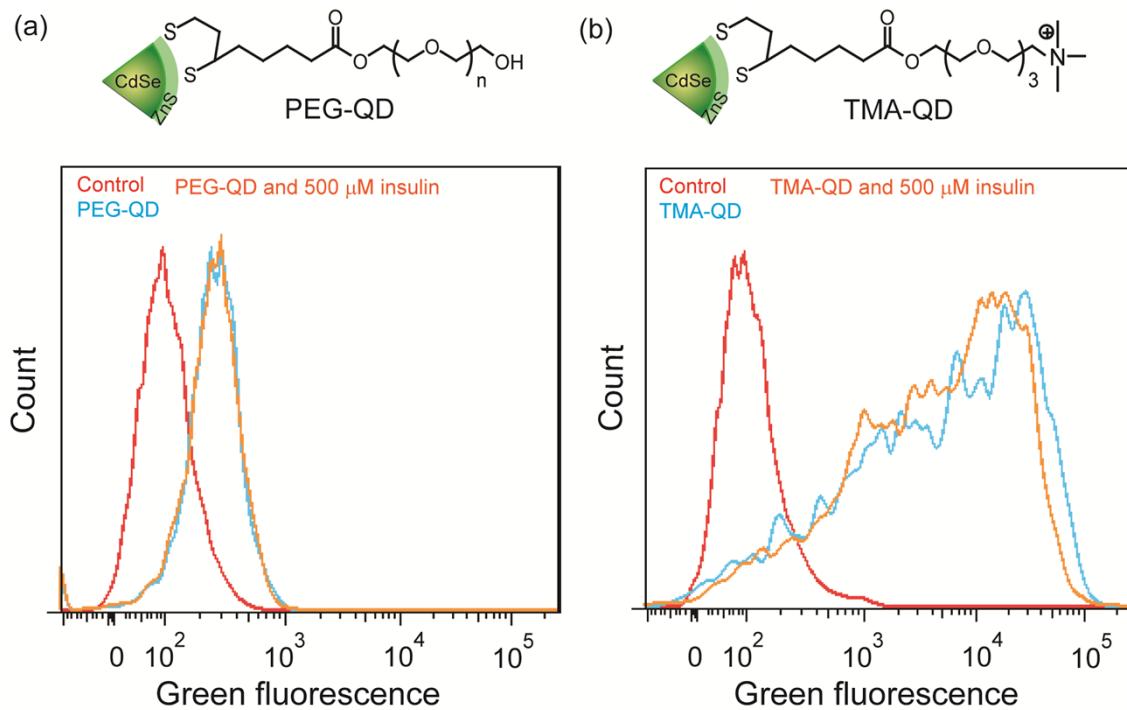


Fig. S3 Two control experiments were performed for the insulin effect on regulating QD uptake. (a) Polyethylene glycol-functionalized QDs (PEG-QDs) and (b) trimethylammonium-functionalized QDs (TMA-QDs) were incubated in differentiated C2C12 cells for 4 h with the treatment of insulin. The concentration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