**SUPPORTING INFORMATION**

Biotinylated Glyco-Functionalized Quantum Dots: Synthesis, Characterization, and Cytotoxicity Studies

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**Experimental Section**

**Materials.** D-Biotin, D-(+)-gluconic acid δ-lactone, N-hydroxysuccinimide (NHS), Ethylenediamine, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), 1,3-Dicyclohexylcarbodiimide (DCC), and ethylenediamine dihydrochloride were purchased from Sigma-Aldrich and used as received. 2-Aminoethyl methacrylamide hydrochloride (AEMA) was synthesized as previously described (1). 4,4’-Azobis(4-cyanovaleric acid) (ACVA, 97%) was purchased from Acros Organics and used as received. N,N’-Dimethylformamide (DMF), isopropanol, methanol, hydroquinone, 1,4-dioxane, and other chemical solvents were purchased from Caledon Chemicals and used as received. Doubly distilled de-ionized water was used in all experiments, and PBS buffer solution (pH 7.4) was used.

**Synthesis of Biotinyl-N-hydroxysuccinimide Ester (Biotin-NHS)**

D-Biotin (2.00 g, 8.19 mmol) and N-hydroxysuccinimide (0.94 g, 8.19 mmol) were dissolved into hot anhydrous DMF (60 mL, 70 °C) in a 100 mL round-bottom flask with
stirring. 1,3-Dicyclohexylcarbodiimide (DCC, 2.19 g, 10.65 mmol) was added, and the solution was stirred overnight at room temperature. The formed dicyclohexylurea was filtered off; most of the solvents were evaporated, and the residue precipitated into excess ether. The white precipitate was filtered and washed with isopropanol three times to give a white powder, and this solid was recrystallized in isopropanol. The final yield of the product is 85%. Finally, its structure was confirmed via $^1$H NMR spectroscopy as shown in Figure S1b.

$^1$H NMR in $d_6$-DMSO, $\delta$ (ppm): 4.1 and 4.3 [m, 2H, (C$_5$H$_8$NH)$_2$CO], 3.1(m, 1H, SCH), 2.7-2.9 (d and s, 6H, SCH$_2$ and CH$_2$CON), 2.6(t, 2H, CH$_2$COO), 1.0-1.7(m, 6H, CHCH$_2$CH$_2$CH$_2$).

**Synthesis of N-(2-Aminoethyl) Biotinamide (Biotin-NH$_2$)**

Ethylenediamine (150 uL, 2.2 mmol), triethylamine (1.5 mL), and DMF (10 mL) were added into a round-bottom 50 mL flask fitted with a stirrer. A DMF solution (5 mL) of biotin-NHS (0.75 g, 2.2 mmol) was added dropwise at room temperature under nitrogen atmosphere and stirred overnight. The formed precipitate was filtered off, most of the solvents were evaporated, and the residue precipitated into excess hexane. The white precipitate was filtered and washed with isopropanol three times to give a white powder. The final yield of the product is 70%. $^1$H NMR in $d_6$-DMSO as shown in Figure S1c, $\delta$ (ppm): 4.1 and 4.3 [m, 2H, (CH$_2$NH)$_2$CO], 2.9-3.2(m, 3H, SCH and CONHCH$_2$), 2.8(d and dd, 2H, SCH$_2$), 2.6(t, 2H, CH$_2$NH$_2$), 2.0(t, 2H, CH$_2$CONH), 1.0-1.7(m, 6H, CHCH$_2$CH$_2$CH$_2$). ESI-MS: calcd. For (C$_{12}$H$_{22}$N$_4$O$_2$S + H)$^+$: 287.4; found: 287.2.

**Synthesis of N-(2-Aminoethyl) Gluconamide Hydrochloride (Sugar-NH$_2$)**

Ethylenediamine (5.5 mL, 0.08 mol) was added to an ethylenediamine dihydro-
chloride solution (12.76 g, 96 mmol, in 100 mL distilled water) in a round-bottom flask fitted with a stirrer. After 1 h of stirring, aqueous solution (70 mL) of D-(+)-Gluconic acid δ-lactone (14.25 g, 0.08 mol) was added dropwise into the above solution cooled in an ice bath condition. The mixture was stirred at room temperature overnight. Finally, the solvent was removed under vacuum; the crude product was washed with isopropanol twice. The product was dissolved into methanol and precipitated into isopropanol twice; the viscous product was dried under vacuum. The final yield of the product is 90%. \(^1\)H NMR in D\(_2\)O, δ (ppm): 4.35(d, 1H), 4.1(t, 1H), 3.5-3.85 (m, 6H), 3.1-3.2(t, 2H). ESI-MS: calcd. For (C\(_8\)H\(_{19}\)N\(_2\)O\(_6\)Cl): 274.7; found: 275.

**Synthesis of Biotinyl-2-Aminoethyl Methacrylamide Hydrochloride (BAEMA)**

Biotin-NHS (0.85 g, 2.5 mmol), hydroquinone (0.05 g), triethylamine (0.7 mL), and 2-aminoethyl methacrylamide hydrochloride (AEMA, 0.41 g, 2.5 mmol) were dissolved in 15 mL anhydrous DMF. This solution was stirred overnight at room temperature, the solid formed was filtered off and DMF was evaporated under reduced pressure. The residue was precipitated into ether and was washed with isopropanol three times. The powder was dried overnight under vacuum. The yield is 70%. \(^1\)H NMR in d\(_6\)-DMSO as shown in Figure S1d, δ (ppm): 5.3 and 5.6(s, 2H, CH\(_2\)CCH\(_3\)), 4.1 and 4.3 [m, 2H, (CHNH)\(_2\)CO], 2.9-3.2(m, 5H, SCH, CONHCH\(_2\), and CH\(_2\)CH\(_2\)NH), 2.8(d and dd, 2H, SCH\(_2\)), 2.0(t, 2H, CH\(_2\)CONH),, 1.7 (s, 3H, CH\(_2\)CCH\(_3\)), 1.0-1.7(m, 6H, CHCH\(_2\)CH\(_2\)CH\(_2\)). ESI-MS: calcd. For (C\(_{16}\)H\(_{36}\)N\(_3\)O\(_4\)S + Na\(^+\)): 377.5; found: 377.2.
Synthesis of Gluconamidoethyl Methacrylamide Hydrochloride (GAEMA)

D-(-)-Gluconic acid δ-lactone (1.77 g, 0.08 mol) and hydroquinone (0.05 g) were dissolved in methanol at 50 °C and then cooled to room temperature before the addition of 2-aminoethyl methacrylamide hydrochloride (AEMA) (2.0 g, 0.012 mmol) and triethylamine (10 mL). The mixture was stirred overnight at room temperature, concentrated by rotary evaporation, and precipitated into isopropanol. The white solid formed was filtered, washed with isopropanol three times, and dried under vacuum. The final yield of the product was 95%. \(^1\)H NMR (D\(_2\)O, ppm): 5.7(s, 1H), 5.4(s, 1H), 4.35(d, 1H), 4.1(t, 1H), 3.6-3.85 (m, 4H), 3.4 (t, 4H), 1.85 (s, 3H).

Characterization

1. Characterization of small reagents. The characteristic signals of biotin, biotin-NHS, and biotin-NH\(_2\) were shown in Figure S1, and the characteristic signals of succinimide group at δ 2.8 ppm could be detected in the \(^1\)H NMR spectrum of biotin-NHS (shown in Figure S1b), and subsequently disappear after reaction with ethylenediamine (shown in Figure S1c). From the peak integration, the purities of both desired products could be calculated to be above 95%. The purity of biotin-NH\(_2\) was further confirmed by mass spectrum (shown in Figure S2 in supporting information).

Similarly, the sugar bearing terminal amino group (Sugar-NH\(_2\)) was synthesized by the reaction of D-(+)-Gluconic acid δ-lactone with ethylenediamine, and the chemical structure was confirmed by mass spectrum (shown in Figure S3).
BAEMA monomer was synthesized by the reaction of biotin-NHS with AEMA, it’s structure was confirmed via \(^1\)H NMR spectroscopy (shown in Figure S1c). The characteristic signals of double bond at \(\delta\) 5-5.5 ppm of BAEMA are clearly evident, and the signal of succinimide group for biotin-NHS at \(\delta\) 2.8 ppm also disappear after being replaced by AEMA. The purity of obtained BAEMA monomer could be calculated to be above 95% based on integration of the peaks at \(\delta\) 5-5.5 ppm and \(\delta\) 4.0 ppm, and the purity was further confirmed also by mass spectrum shown in Figure S4.

2. Characterization of biotinylated glycopolymers. The final statistical glycopolymer was obtained and analyzed by \(^1\)H NMR in D\(_2\)O solvent. The GAEMA monomer conversion is about 80% in this polymerization condition, and the DP\(_n\) of GAEMA and AEMA could be calculated to be 36 and 24, respectively, from their characteristic signals at \(\delta\) 3-4 ppm shown in Figure S5a. However, the signals of BAEMA could not be clearly detected or quantified by \(^1\)H NMR as the characteristic peaks of BAEMA were masked. The amount of biotin contents in the glycopolymer was therefore further quantified to 4.4 using HABA/avidin binding assay. By dissolving the glycopolymer into water and adding into the HABA/avidin solution, the change of absorbance of HABA/avidin complex at 500 nm was monitored using UV-vis absorption spectra. The obtained glycopolymer was thus denoted P(GAEMA\(_{36}\)-stat-AEMA\(_{24}\)-stat-BAEMA\(_{4.4}\)). Aqueous gel permeation chromatography was used also to analyze the polymer, and a mono-modal peak with a very low polydispersity index was obtained as shown in Figure S5b. The \(M_n\) and \(M_w/M_n\) were determined to be 12,900 and 1.19, respectively. By comparing to the theoretical molecular weight
($M_{n,\text{theo}} = 18,130$) calculated from the conversion using $^1$H NMR spectroscopy, the experimental $M_n$ is found to be smaller and this is attributed to the fact that a conversion GPC calibration was used using polyethylene oxide as standards.

Reference.

Figure S1. $^1$H NMR spectra of biotin (a), Biotin-NHS (b), Biotin-NH$_2$ (c), and BAEMA (d) in d$_6$-DMSO solvent. The zoom region from δ 2-3.5 ppm is shown in subset.
**Figure S2.** Mass spectrum for N-(2-Aminoethyl) Biotinamide (Biotin-NH₂).

**Figure S3.** Mass spectrum for Gluconamidoethylamide Hydrochloride (Sugar-NH₂).
**Figure S4.** Mass spectrum for Biotinyl-2-Aminoethyl Methacrylamide Hydrochloride (BAEMA).
Figure S5. Assigned \(^1\)H NMR spectrum (a) and aqueous GPC trace (b) of the P(GAEMA-stat-AEMA-stat-BAEMA) copolymer. It should be noted that the characteristic signals of BAEMA are undistinguishable for its very low contents in glycopolymer.