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Probing Lectin and Sperm with Carbohydrate-Modified Quantum Dots

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We report the encapsulation of quantum dots with biologically important β-N-acetylglucosamine (GlcNAc) in different ratios, together with studies of their specific/sensitive multivalent interactions with lectins and sperm by fluorimetry, transmission electron microscopy, dynamic light scattering microscopy, confocal imaging techniques, and flow cytometry. These GlcNAc-encapsulated quantum dots (QDGLNs) specifically bind to wheat germ agglutinin, and cause fluorescence quenching and aggregation. Further studies of QDGLNs and the mannose-encapsulated QDs (QDMANs) with sperm revealed site-specific interactions, in which QDGLNs bind to the head of the sperm, while QDMANs spread over the whole sperm body.

Introduction

Nanoparticles have attracted much attention in the fields of sensitizers, carriers, supporters, spectroscopic enhancers, and probes for biomedical applications,[1] because they exhibit size-dependent optical and electronic properties[2,3] as well as possible multivalent effects with biomolecules.[4] In particular, quantum dots (QDs) containing semiconductor materials have been extensively exploited for sensing metal ions and biomolecules,[2] among which carbohydrate–protein interactions are of prime interest. Carbohydrate–protein interactions play a crucial biological role in cellular recognition, endocytosis, fertilization, cell adhesion, and information transfer,[5] in which carbohydrates show enormous structural and functional diversity upon interacting with proteins. Because of the weakness of monovalent interactions, numerous carbohydrate carriers, such as dendrimers, polymers, liposomes, and nanoparticles, have been developed to take advantage of multivalency.[4] QDs encapsulated with WGA (wheat germ agglutinin) have been shown to retain their specificity for the cell walls of Gram-positive bacteria,[4o] though low solubility shortens the useful lifespans of the conjugates.

At the cellular recognition site, β-*N*-acetylglucosamine (GlcNAc) plays a significant role, especially in fertilization processes.[6] The GlcNAc unit is an essential component of the ZP3 protein on an egg membrane (*zona pellucida*) that facilitates binding with the complementary sugar-binding enzyme (e.g., galactosyltransferase) on the surface of the sperm.[6] Here we demonstrate the QDs encapsulated with the biologically important GlcNAc and their specific/sensitive multivalent interactions with sperm and WGA, a plant lectin isolated from *Triticum vulgaris* and possessing two recognition sites for simultaneously binding two GlcNAc moieties.[7]

Results and Discussion

High-quality CdSe/ZnS core-shell QDs were prepared by the previously reported one-pot procedure,[8a] with slight modification (see the Experimental Section). Pyridine was used to replace trioctylphosphine oxide (TOPO) on the surfaces of the QDs, without affecting the quality of nanocrystals. This pyridine encapsulation provides better access for organic modifiers to the surfaces of nanoparticles.[8b] Disulfide **4**, bearing GlcNAc moieties, was obtained by glycosidation of the protected GlcNAc derivative **1** with 11-acetylthioundecan-1-ol (**2**), followed by saponification and autoxidation (Scheme 1). The pyridine-encapsulated QDs were then treated with disulfide **4** and $NaBH_4$ in aqueous solution to give the water-soluble GlcNAc-encapsulated QDs (QDGLNs).

After being rinsed with anhydrous MeOH/Et_2O (1:1) and dried in vacuo, the purified QDGLN nanoparticles were characterized by UV-visible, fluorescence, FTIR, and ^{1}H NMR spectroscopy and by transmission electron microscopy (TEM). The mean diameter of the core-shell as measured by TEM is about 5 nm, and the presence of the carbohydrate moiety was confirmed by the ^{1}H NMR analysis. The absence of any S–H stretching band in the 2650–2450 cm^{-1} region in the FTIR confirmed the formation of the QD–sulfur bond. The prepared

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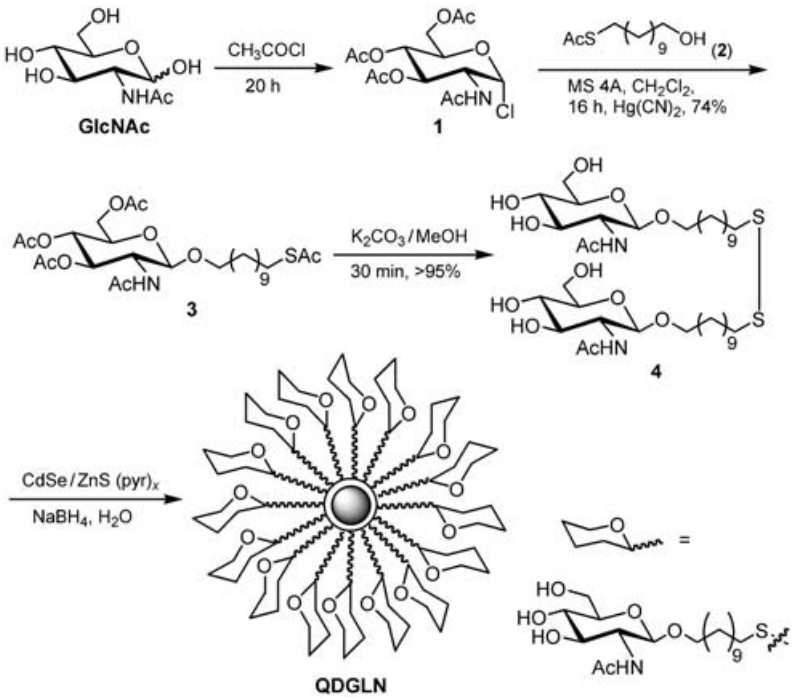
QDGLN nanoparticles were found to be both thermally and photochemically stable, exhibiting strong fluorescence with quantum yields (Φ_f) of $\approx$0.35 in deionized water as well as in phosphate buffer at pH values of 6–8. The concentration of CdSe/ZnS QDs was estimated by using a reported procedure,[9] and the number of GlcNAc moieties on each QD particle was quantitatively determined by a chemical method with pentane-2,4-dione and Ehrlich's reagent.[10] As a result, 210 GlcNAc units were estimated to be anchored on each QD, in agreement with the previous report for carbohydrate-encapsulated 6 nm gold nanoparticles.[4k]

In this study, the binding affinity of the QDGLNs was demonstrated by fluorescence titration against unlabeled WGA lectin (Figure 1), with the fluorescence intensity of the QDGLNs in phosphate buffer (pH 6.8) gradually being quenched with increasing lectin concentrations. The relative change in fluorescence intensity ($\Delta F/F_0$) at 604 nm is plotted against the concentration of lectin (inset in Figure 1).[11] The association constant (K_a) of the QDGLNs with WGA, as estimated from the intercept and slope in the linear region of the plot, is on the order of 10^7, $\approx$3–4 orders of magnitude higher than that of the corresponding monomer,[11,12] thus manifesting the great enhancement of binding ability achievable by simultaneous cooperative interactions of multiple GlcNAc residues with lectins. A competitive experiment was performed by adding monomeric GlcNAc, and the results showed the recovery of the fluorescence intensity upon addition of an appreciable amount of GlcNAc (25 mg, for example; not shown here). Excess GlcNAc was also added in an attempt to achieve the recovery of the original fluorescent intensity. Unfortunately, the appearance of turbidity in solution upon addition of $>$150 mg of GlcNAc made this approach infeasible.

Although the actual quenching mechanism still awaits resolution, the decrease in fluorescence lifetime during the titration has prompted us to propose a radiationless deactivation pathway incorporating either an energy-transfer or an electron-transfer process upon complexation. The lack of a chromophore with an absorption energy gap of $>$500 nm in lectin seems to disfavor a tentative mechanism incorporating energy transfer. Alternatively, the quenching is more plausibly attributable to electron transfer from the QDGLNs to the amino acid residues on WGA lectin, forming, for example, a CH–π interaction with tryptophan.[13]



Scheme 1. Preparation of the water-soluble β-GlcNAc-encapsulated quantum dots (QDGLNs).

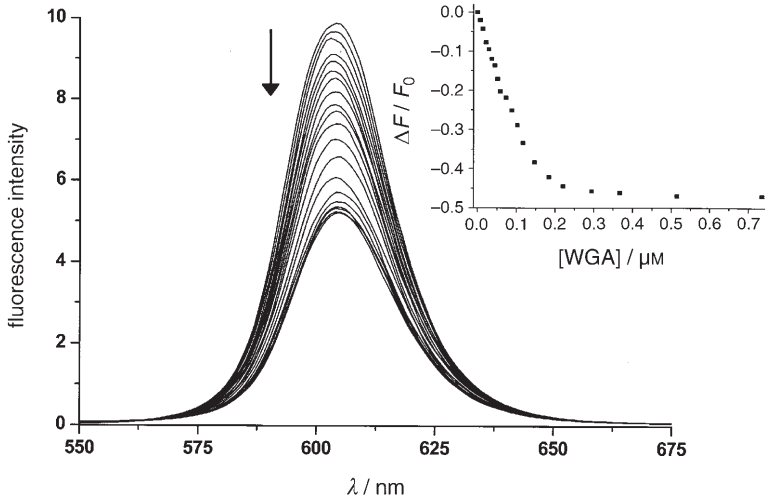


Figure 1. Fluorescence titration spectra of QDGLN (3.8×10^{-8} M) as a function of WGA lectin concentration at concentrations from 0.01 to 0.73 μM. The excitation wavelength is 400 nm. Inset: The relative change in fluorescence intensity ($\Delta F/F_0$) at 604 nm plotted against the concentration of lectin.

Interaction between multivalent receptors and ligands has been studied by light-scattering experiments, fluorescence resonance energy transfer, or TEM.[4i,14] Here, direct support for the QDGLN–WGA interaction is provided by the TEM image (Figure 2). In this approach, WGA was incubated with the QDGLNs in phosphate buffer for 30 min at room temperature and viewed under a microscope, with aggregation of QDGLNs due to multivalent binding with WGA being found. In a control experiment, aggregation of QDGLNs was unconvincing when WGA was replaced by bovine serum albumin (BSA), a protein that cannot efficiently bind with GlcNAc. Note that a regular arrangement pattern resolved from the TEM image in the case of BSA (Figure 2) might simply be the result of the adsorption of BSA on the QDGLNs.

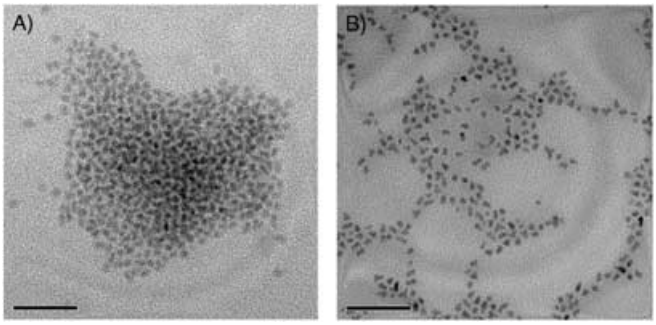


Figure 2. TEM images of QDGLN with A) WGA and B) BSA. Scale bars = 50 nm.

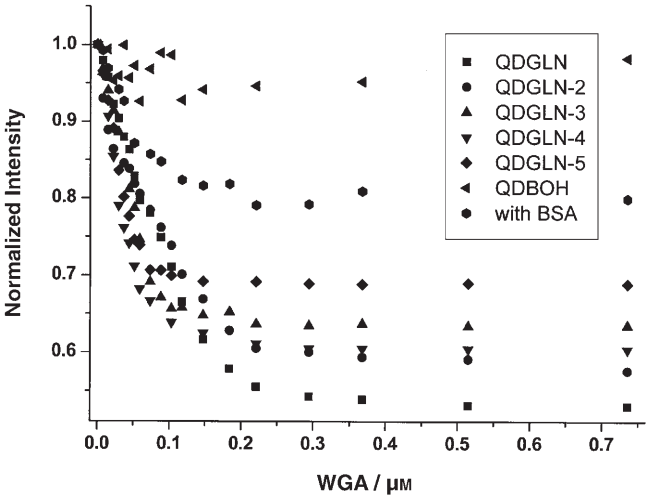


Figure 3. Changes in fluorescence intensity observed on addition of WGA to glycoQDs with different surface modifications (see text).

Aggregation in solution was also investigated by dynamic light scattering (DLS) microscopy. The results estimated the size distributions of native QDGLNs, QDGLN–BSA conjugate, and QDGLN–WGA conjugate to be around 14–16, 27–29 and 240–260 nm, respectively, in agreement with the TEM observation. It is also worth noting that the hydrodynamic radii of the QDs are slightly larger than the TEM radii, possibly due to the weak contrast for the nonmetallic part in the TEM measurement.

A comprehensive study to probe carbohydrate–protein interactions with QDGLNs covered with carbohydrate moieties of various densities was also performed. By a similar synthetic procedure, surface modification of QDs with 11-mercaptoundecyl-GlcNAc (derived from disulfide **4**) and 4-mercaptobutan-1-ol in different ratios—5:1, 3:1, 1:1, and 1:3—gave the hybrid glycoquantum dots QDGLN-2, QDGLN-3, QDGLN-4, and QDGLN-5, respectively. Quantitative analyses showed ratios of 190 (QDGLN-2), 160 (QDGLN-3), 120 (QDGLN-4), and 100 (QDGLN-5) GlcNAc molecules per QD, with the remaining QD surface covered with mercaptobutan-1-ol. In a control experiment, butan-1-ol-encapsulated dummy quantum dots (QDBOHs) were prepared by using 4-mercaptobutan-1-ol as the sole surface modifier. The binding affinities of WGA with these GlcNAc-modified QDs were examined under the same conditions. A similar trend in fluorescence quenching was observed during the titrations (Figure 3); however, the quenching power decreased as the density of GlcNAc moieties decreased (with K_a on the order of 10^6). Conversely, the QDBOHs showed no interaction with WGA and consequently caused negligible quenching of the fluorescence. Note that the QDGLNs showed a tiny but non-negligible decrease in fluorescence upon titration with BSA, possibly due to the adsorption of BSA on QDs as indicated by the TEM image (Figure 2).

Several reports have shown that the sugar density on a glycoconjugate should be lower than 100% in order to achieve optimum activity.[15] However, the maximum activity depends on the area of the sugar molecule, and the binding efficiency remains unchanged even when the loading is high.[15a,b] In our case, high loading caused similar binding constants of the order of 10^6–10^7. In addition, we noticed a moderate decrease in the activity as the sugar density decreased (see Figure 3). It seems that the quenching of QDs is due either to the binding of lectin or to lectin denaturation resulting from the hydrophobic interaction between the linker and the hydrophobic moiety of lectin. Apparently, knowledge of the surface area of sugar and the distance between two binding sites (22–42 Å) of WGA lectin[16a] is pivotal for dealing with the lectin–sugar interaction. The radius of a QDGLN is estimated from DLS microscopy as 5.5 nm, so an effective surface area of 190 Å^2 can be deduced for each sugar molecule. The size of lectin ($4\times4\times7$ nm)[16b] is much larger than that of a sugar molecule, so it is quite unlikely that lectin might enter into the sugar moieties to cause denaturation through hydrophobic interaction with linkers. We thus conclude that the variation in the quenching ability is due to the difference in the amount of electron transfer between the QDs and the lectin.

It has been proposed that GlcNAc-conjugated proteins may initiate the sperm acrosomal reaction in mice.[6] In contrast, monovalent GlcNAc failed to do so, so glycoQDs may also serve as ideal probes for investigating the distribution of complementary binding proteins on the surface of sperm. We thus examined the binding capacity, and hence the effect on the acrosomal reaction, by using GlcNAc-encapsulated QDs with fresh sperm obtained from mice, pigs, and sea-urchins. In this approach, the fresh sperm ($\approx3\times10^7$) were incubated with QDGLNs (0.2 mg) in PBS buffer (pH 7.4) for 30 min, and the unbound QDGLNs were washed away by centrifugation. Confocal microscopy images showed QDGLN nanoparticles concentrated at the sperm heads (Figure 4A and B for sea-urchin sperm). In contrast, mannose-encapsulated quantum dots (QDMANs) tended to spread over the whole sperm body under similar incubation conditions (Figure 4C for mouse sperm). These results can be tentatively interpreted in terms of the different distributions of the GlcNAc and Man receptors on the sperm surface.

The binding capability of QDGLNs with live sperm was detected by flow cytometry with use of a monitor gated at 25 μm for the effective size of the live sperm. As shown in Figure 5A, addition of QDGLNs in increments caused gradual shifts of the signal along the *x*-axis, indicating fluorescence

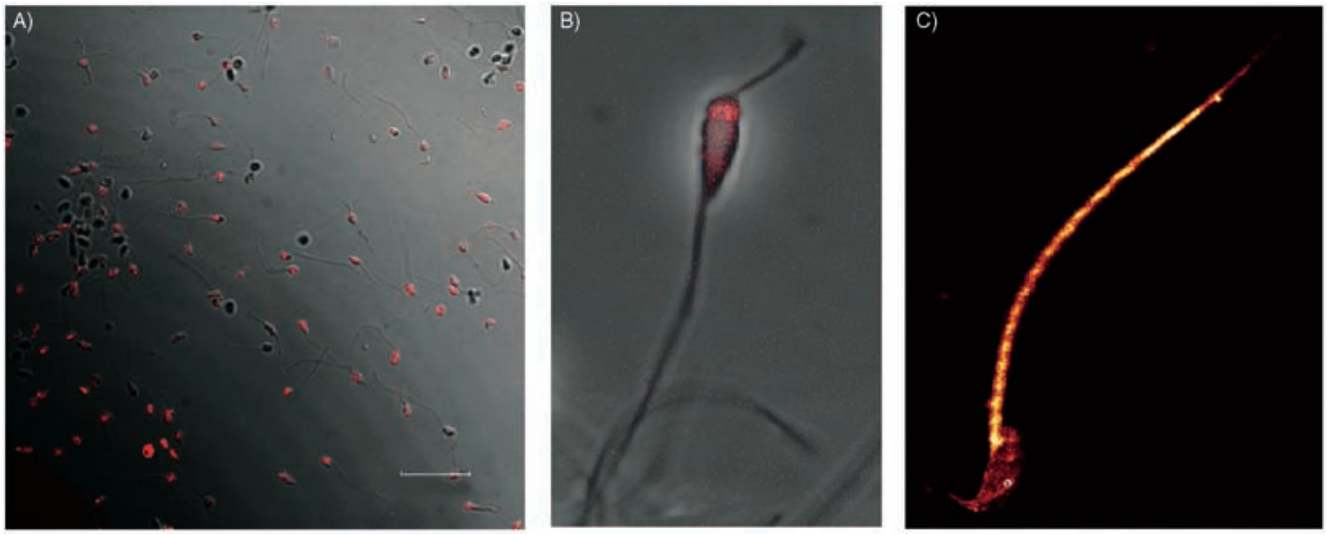


Figure 4. Confocal microscope imaging for staining of sperm with glycoquantum dots: A) selective QDGLN labeling on the heads of sea-urchin sperm (scale bar = 20 μm), B) close-up of QDGLN-labeled sea-urchin sperm, and C) close-up of QDMAN-labeled mouse sperm.

changes on the pig sperm due to the binding with fluorescent QDGLNs. Unbound QDGLN nanoparticles would not interfere with the observed fluorescence enhancement, because they would be too small (< 10 nm) to appear in the monitor gate (25 μm).

Incubation of live sea-urchin sperm with QDGLNs in artificial seawater (ASW) also showed a similar fluorescence enhancement on flow cytometry (Figure 5B). Conversely, the increase in fluorescence intensity was less obvious when sea-urchin sperm were incubated with QDGLNs in egg water (Figure 5C). Egg water is an extract of egg jelly coat, which contains the GlcNAc-conjugated proteins and other biomaterials for binding with sea-urchin sperm to trigger the acrosomal reaction.[6] The acrosome on the sperm head will fall off during this event. The binding of QDGLNs to sea-urchin sperm might be inhibited by the GlcNAc-conjugated proteins present in egg water, thus accounting for the relatively small fluorescence increase (Figure 5C) in comparison with that in ASW (Figure 5B).

In conclusion, water-soluble multivalent glycoQDs have been prepared in a simple way in order to study the specificity and sensitivity of their multivalent interaction with lectins. The reported binding constants corroborate results obtained previously,[11,12] while the high fluorescence yield and multivalent effect illustrate the superiority of the QDGLN approach. The results are illustrated by the ability of the QDGLNs to bind with sperm and possibly influence the acrosomal reaction, so that one can follow the dynamic changes in the fertilization process.[6] Moreover, the glycoQDs might also have potential for exploration of the carbohydrate–protein interaction through the surface plasmon enhanced fluorescence spectroscopy (SPFS) technique.[12e] We thus believe that the GlcNAc-derivatized multivalent scaffold could spark broad interest in the fields of fundamental biochemistry and biosensors.

Experimental Section

General methods: All experiments requiring anhydrous conditions were performed under argon. All solvents and reagents were reagent grade and were used without further purification. Diethyl ether and toluene were distilled from Na/benzophenone, methanol was distilled from magnesium turnings, and CH_2Cl_2 was distilled from CaH_2. Infrared (IR) spectra were recorded on a Nicolet Magna 550-II spectrometer. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR and DEPT) spectra were recorded in Varian Unity Plus-400 (400 MHz) and Bruker Avance-400 FT-NMR spectrometers; chemical shifts are reported in δ units relative to tetramethylsilane (TMS) with residual protons in the solvent as an internal standard: $CDCl_3$, $\delta = 7.24$ (for ^{1}H NMR) and $\delta = 77.0$ (for ^{13}C NMR and DEPT); CD_3OD $\delta = 3.31$ (for ^{1}H NMR) and $\delta = 49.15$ (for ^{13}C NMR and DEPT); [D_6]DMSO, $\delta = 2.49$ (for ^{1}H NMR) and $\delta = 39.5$ (for ^{13}C NMR and DEPT)). Mass spectra (MS) and high-resolution mass spectra (HRMS) were measured with JEOL JMS-HX 110 or JEOL SX-102A spectrometers. Thin-layer chromatography (TLC) was performed on Merck ART.5544 precoated sheets, and TLC results were viewed by UV lamp. Chromatography was performed under gravity on silica gel 60 of 0.040–0.063 mm particle size. Elemental analysis was carried out on Perkin–Elmer CHN-2400 II or Heraeus Vario EL III elemental analysis instruments at the Instrumentation Center, National Taiwan University. Absorption spectra were recorded on a JASCO V-530 UV/Vis spectrometer. Emission spectra were recorded with a JASCO FP-6200 spectrofluorimeter and an AMINCO/Bowman Series 2 spectrometer. Transmission electron microscopy images were obtained with a Philips/FEI Tecnai 20 G2 S-Twin transmission electron microscope. The fixed sperm were viewed under a Leica TSCspl2 confocal microscope. Dynamic light scattering microscopy was conducted with a PD2010/DLS laser light scattering detector.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (1): 2-Actetamido-2-deoxy-α-D-glucopyranose (GlcNAc, 5 g, 22.6 mmol) was stirred with freshly distilled acetyl chloride for 24 h under argon. After addition of CH_2Cl_2, the organic layer was separated, and washed with water (4 × 20 mL), aqueous $NaHCO_3$ (2 × 20 mL), and brine solutions (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to provide the practically pure product **1**, which was used directly in the next re-

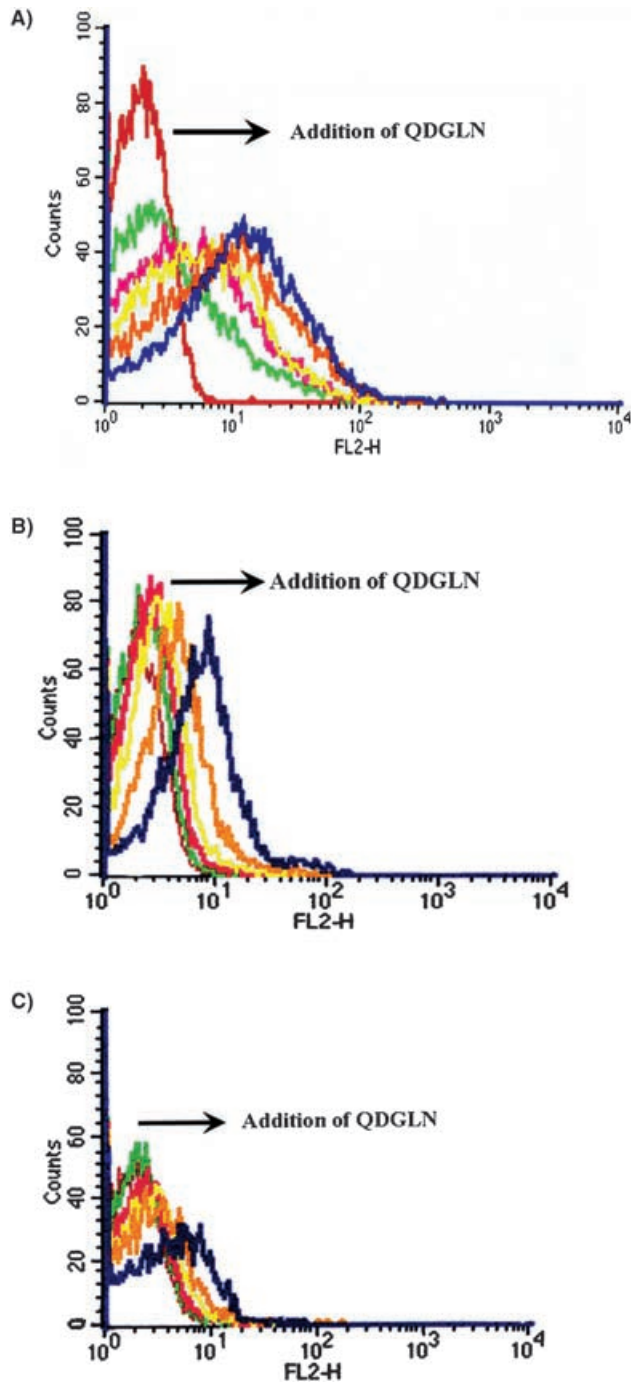


Figure 5. Flow cytometric measurements for binding of live sperm with QDGLN nanoparticles: A) pig sperm (90 000 mL^{-1}) in PBS buffer, B) sea-urchin sperm in artificial sea water, and C) sea-urchin sperm in egg water with addition of QDGLN (2, 4, 6, 10, and 20 ng); $\lambda_{ex}=488$ nm.

action. $C_{14}H_{20}ClNO_8$; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=6.13$ (d, $J=3.7$ Hz, 1H), 6.03 (d, $J=8.7$ Hz, 1H), 5.26 (t, $J=10.0$ Hz, 1H), 4.80 (m, 1H), 4.51–4.46 (m, 1H), 4.25–4.04 (m, 3H), 2.03 (s, 3H), 1.97 (s, 6H), 1.92 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=170.4$ (C), 170.1 (C), 170.0 (C), 168.7 (C), 93.4 (CH), 70.3 (CH), 69.4 (CH), 66.9 (CH), 60.7 (CH_2), 52.7 (CH), 22.2 (CH_3), 20.1 ($2CH_3$), 20.0 ppm (CH_3).

11-Thioacetylundecan-1-ol (2): A solution of 11-bromoundecan-1-ol (4.16 g, 16.6 mmol) and KSAc (2.85 g, 25 mmol) in DMF (25 mL) was stirred for 1 h at 90 °C. The residue obtained on concentration was dissolved in CH_2Cl_2 and washed with water. The organic phase was dried ($MgSO_4$), concentrated, and purified by silica gel column chromatography with elution with EtOAc/hexane (2:8) to yield compound **2** (3.82 g, 96%). $C_{13}H_{26}O_2S$: ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=3.57$ (t, $J=6.6$ Hz, 2H), 2.82 (t, $J=7.4$ Hz, 2H), 2.27 (s, 3H), 1.51 (m, 4H), 1.32–1.22 ppm (m, 14H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=$ 196.1 (C), 62.9 (CH_2), 32.7 (CH_2), 30.5 (CH_3), 29.5 (CH_2), 29.4 ($2CH_2$), 29.3 ($2CH_2$), 29.1 (CH_2), 29.0 (CH_2), 28.7 (CH_2), 25.7 ppm (CH_2); FAB-MS *m/z* (rel intensity) 247 $[M+H]^+$ (100); HRMS calcd for $C_{13}H_{27}O_2S$ $[M+H]^+$: 247.1732; found 247.1734.

11-Thioacetoxyundecyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3): A mixture of glycosyl chloride **1** (1.75 g, 4.8 mmol) and thioacetylundecanol **2** (1.44 g, 5.86 mmol) in toluene/acetonitrile (20 mL 1:1 *v/v*) was stirred at room temperature for 16 h in the presence of Drierite (1.5 g) and $Hg(CN)_2$ (2.5 g). The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL) and washed with brine (25 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated, and purified by silica gel column chromatography with elution with EtOAc/hexane (1:1). $C_{27}H_{45}NO_{10}S$: Yield 2.05 g (74%). TLC (EtOAc/hexane (1:1)) $R_f=0.20$; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta=5.51$ (d, $J=8.6$ Hz, 1H), 5.28 (t, $J=9.5$ Hz, 1H), 5.03 (t, $J=9.5$ Hz, 1H), 4.65 (d, $J=8.3$ Hz, 1H), 4.22 (dd, $J=12.2$, 4.7 Hz, 1H), 4.10 (dd, $J=7.3$, 2.4 Hz, 1H), 3.83–3.76 (m, 2H), 3.69–3.64 (m, 1H), 3.46–3.42 (m, 1H), 2.83 (t, $J=7.3$ Hz, 2H), 2.30 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.92 (s, 3H), 1.70–1.51 (m, 6H), 1.32–1.22 ppm (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta=196.1$ (C), 170.8 (C), 170.7 (C), 170.1 (C), 169.4 (C), 100.6 (CH), 72.3 (CH), 71.6 (CH), 69.9 (CH_2), 68.6 (CH), 62.1 (CH_2), 54.8 (CH_2), 30.5 (CH_2), 29.5 ($2CH_2$), 29.4 ($2CH_2$), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.7 (CH_2), 25.7 (CH_2), 23.2 (CH_3), 20.7 (CH_3), 20.6 ppm ($2CH_3$); IR (KBr): $\tilde{\nu}=$ 2927, 2855, 1747, 1692, 1663, 1544 cm^{-1}; FAB-MS *m/z* (rel intensity) 576 $[M+H]^+$ (70); HRMS calcd for $C_{27}H_{46}NO_{10}S$ $[M+H]^+$: 576.2842; found 576.2845.

11-Mercaptoundecyl 2-acetamido-2-deoxy-β-D-glucopyranoside dimer (4): Compound **3** (200 mg) was stirred with K_2CO_3 (catalytic amount) in anhydrous MeOH at ambient temperature until TLC analysis confirmed the completion of the reaction. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with elution with MeOH/CH_2Cl_2 (1:9) to give disulfide **4**. $C_{38}H_{73}N_2O_{12}S_2$: Yield 136 mg (96%). TLC (MeOH/CH_2Cl_2 (1:9) $R_f=$ 0.10; ^{1}H NMR (CD_3OD, 400 MHz): $\delta=4.37$ (d, $J=8.0$ Hz, 2H), 3.95-3.83 (m, 4H), 3.75 (dd, $J=12.2$, 4.7 Hz, 2H), 3.62 (dd, $J=7.3$, 2.4 Hz, 2H), 3.55–3.12 (m, 10H), 2.46 (m, 4H), 1.97 (s, 8H), 1.62–1.47 (m, 10H), 1.45–1.23 ppm (m, 30H); ^{13}C NMR (CD_3OD, 100 MHz): $\delta=$ 173.7 (2C), 102.9 (2CH), 77.9 (2CH), 76.0 (2CH), 72.0 ($2CH_2$), 70.8 (2CH), 63.3 (2CH), 62.5 ($2CH_2$), 57.2 ($2CH_2$), 39.0 (CH_2), 30.9 ($2CH_2$), 30.8 ($2CH_2$), 30.7 ($2CH_2$), 30.6 ($2CH_2$), 30.5 ($4CH_2$), 30.3 ($2CH_2$), 27.1 (CH_2), 26.2 (CH_2), 23.8 (CH_2), 23.1 ppm ($2CH_3$); IR (KBr): $\tilde{\nu}=3387$, 2927, 2853, 1654, 1559 cm^{-1}; FAB-MS *m/z* (rel intensity) 813 $[M+H]^+$ (5); HRMS calcd for $C_{38}H_{73}N_2O_{12}S_2$ $[M+H]^+$: 813.4605; found 813.4627.

Synthesis of semiconductor quantum dots: A mixture of cadmium oxide (0.0386 g), trioctylphosphine oxide (5.6652 g), and hexadecylamine (2.8326 g) was dried in vacuum for 2 h and heated to 340 °C under argon to obtain a colorless solution. A solution of selenium (0.025 g) in tributylphosphine (1 mL) was added to the colorless solution at 230 °C, and the mixture was stirred for 20 min at the same temperature. A mixture of diethylzinc (1.1 mL of 15% solution in hexane) and bis(trimethylsilyl) sulfide (133.5 μL) in tributyl-

phosphine (4.3 mL) was added slowly at 180 °C with a flow rate of 0.1 mL min^{-1}. The mixture was stirred at 180 °C for 10 min and at 100 °C for 1 h. The reaction mixture was cooled and dissolved in chloroform, and MeOH was added to precipitate semiconductor quantum dots. The precipitate was washed twice with MeOH, and dried in vacuum for 16 h.

Synthesis of GlcNAc-encapsulated quantum dots (QDGLNs): A solution of the TOPO-capped quantum dots (20 mg) was heated in dry pyridine (10 mL) at 70 °C for 12 h. The mixture was cooled, excess pyridine was removed under reduced pressure, and the pyridine-capped quantum dots were coevaporated twice with toluene. A suspension of the pyridine-capped quantum dots in toluene (8 mL) was stirred with disulfide **4** (100 mg) and $NaBH_4$ (10 mg) in water (8 mL) at 60 °C for 5 h. The reaction mixture was cooled and subjected to centrifugation (9000 rpm for 10 min). The aqueous layer was separated and concentrated, and the sugar-encapsulated nanoparticles were precipitated by addition of MeOH/Et_2O (20 mL, 1:1). The precipitates were washed with MeOH/Et_2O (1:1, 4 mL × 3), and dried in vacuo for 16 h to provide the high-quality GlcNAc-encapsulated QDs (QDGLNs, 15 mg).

Synthesis of butan-1-ol-encapsulated quantum dots (QDBOHs): The pyridine-capped quantum dots were stirred with 4-mercaptobutan-1-ol in toluene by a procedure similar to that used for the QDGLNs, to give the butan-1-ol-encapsulated QDs.

Synthesis of hybrid quantum dots modified with GlcNAc and butan-1-ol in different ratios: The pyridine-capped quantum dots were treated with disulfide **4** and 4-mercaptobutan-1-ol, in the ratios indicated (5:1, 3:1, 1:1, or 1:3), by a procedure similar to that used for the QDGLNs, to give the hybrid QDs.

Quantification of the GlcNAc moieties on each quantum dot: Chemical analysis of glucosamine was carried out by the method developed by Elson and Morgan.[10] Ehrlich's reagent was prepared by dissolving *p*-dimethylaminobenzaldehyde (1.6 g) in HCl (12 M, 30 mL) and EtOH (96%, 30 mL). A solution of acetylacetone (pentane-2,4-dione, 0.75 mL) in aqueous sodium carbonate (1.25 M, 25 mL) was prepared. An aqueous solution of glucosamine hydrochloride (2 mL of known concentration) was heated at 96 °C with the freshly prepared acetylacetone solution (2 mL) for 20 min. The reaction mixture was allowed to cool to room temperature and treated with Ehrlich's reagent (2 mL) and EtOH (96%, 20 mL) at room temperature for 1 h. The resulting red solution showed an absorption maximum at 527 nm (see the Supporting Information). A calibration line was derived from a series of similar experiments with different concentrations of glucosamine to give the value of 23 500 $cm^{-1}\,M^{-1}$ for the extinguish coefficient (ε).

The GlcNAc-encapsulated quantum dots (2.3 mg) were heated with HCl (2 M) at 100 °C in a sealed tube for 14 h to release the glucosamine moieties (HCl salt form) from the nanoparticles. The glucosamine layer was cooled, neutralized with NaOH (4 M), and made up to a 10 mL solution. An aliquot (2 mL) of solution was taken, and subjected to quantification analysis for the content of glucosamine by the procedure described above.

Binding study of the GlcNAc-modified quantum dots with WGA lectin: An aliquot of QDGLNs (50 μL of 1 mg mL^{-1} aqueous solution) was adjusted with phosphate buffer (10 mM, pH 6.8) to make a 2 mL solution, which was placed in a quartz cuvette (1 cm width) at 293 K for measurements.

WGA lectin (1.47×10^{-5} M) was added in an incremental fashion (0.11, 0.23, up to 12 equiv). The fluorescence spectra with 400 nm excitation were recorded for each addition. The fluorescence intensity at 604 nm was monitored as a function of lectin concentration. The binding constant (K_a) is derived from Eq. (1).[11]

$$[\text{Lectin}]\,F_0/\Delta F = [\text{Lectin}]\,F_0/\Delta F_{max} + F_0/(\Delta F_{max}\cdot K_a) \quad (1)$$

F is the emission intensity, F_0 is the original emission intensity of the free QDGLNs, and ΔF_{max} is the largest change in emission intensity after saturation with lectin.

Microscopy imaging of the QDGLN-WGA complex: An aliquot of QDGLNs (50 μL of 1 mg mL^{-1} aqueous solution) was incubated with WGA or BSA (1.47×10^{-5} M, 100 μL) in phosphate buffer (10 mM, pH 6.8, 2 mL) for 30 min at room temperature. Approximately 5 μL of sample was placed on a carbon-coated grid, and the TEM imaging was performed under a Philips/FEI Tecnai 20 G2 S-Twin transmission electron microscope. In addition, approximately 1 mL of sample was taken for the DLS microscopy with a PD2010/DLS laser light scattering detector (Precision Detectors).

Microscopy imaging of the labeled sperm with sugar-encapsulated quantum dots: Mouse sperm (100 μL of a 2.7×10^{8} sperm per mL suspension) were incubated with glycoQDs (QDGLNs or QDMANs, 100 μL of 2 mg mL^{-1} solution) in PBS buffer (2 mL, 10 mM, pH 7.4) for 30 min at room temperature. The unbound glycoQD nanoparticles were washed away from sperm by use of PBS buffer (3 × 5 mL) with centrifugation (1500 rpm). The sperm, anchored by glycoQDs, were suspended in PBS buffer (1 mL), and an aliquot (30 μL) was placed on a microscope plate. The sample was kept drying for 10 min, and formaldehyde (1 mL of 2% aqueous solution) was added. After 20 min of fixation, the plate was washed with PBS buffer, and then dried. A few drops of gel were added, and a cover glass was applied. Imaging was performed under a Leica TCSsp2 confocal microscope.

Pig sperm (100 μL of 2.7×10^{7} sperm per mL suspension) and sea-urchin sperm (100 μL of 2.5×10^{10} sperm per mL suspension) were used for the labeling experiment by a procedure similar to that used for mouse sperm.

Flow cytometric analysis of the binding of QDGLN with live sperm

Method A: Fresh pig sperm (ca. 3.5 μL of 2.7×10^{7} sperm per mL suspension) were incubated with various doses of QDGLNs (2, 4, 6, up to 20 ng) in PBS buffer (2 mL, 10 mM, pH 7.4) for 30 min. Unbound nanoparticles were washed away from the sperm by use of PBS buffer (3 × 5 mL) with centrifugation. The QDGLN bound sperm were resuspended in PBS buffer (2 mL), and were subjected to flow cytometric analysis. A FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA) was used to measure the fluorescence intensity with excitation at 488 nm. The monitor gate of 25 μm was set to accommodate the effective size of live sperm. The signal along the *x*-axis indicates the fluorescence intensity, and the height along the *y*-axis indicates the corresponding number (counts) of species showing the fluorescence.

Method B: A solution of KCl (0.5 M) was injected into the coelomic cavity of a sea-urchin to stimulate ejection of sperm. By a procedure similar to that used for pig sperm, the sea-urchin sperm (20 μL of a 2.5×10^{10} mL^{-1} suspension) were incubated with various doses of QDGLNs (2–20 ng), either in artificial sea water (ASW) or in ASW containing egg jelly stripped from unfertilized urchin eggs by passing 5 × through a 85 μM nylon mesh.

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