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Characterization of multivalent lactose quantum dots and its application in carbohydrate–protein interactions study and cell imaging

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ABSTRACT

We have previously reported a facile and convenient method for the preparation of a new type of lactose-CdSeS/ZnS quantum dots conjugates (Lac-QDs) that exhibit biocompatibility, noncytotoxicity and specificity to leukocytes. In order to further study the carbohydrate–protein interactions, a series of Lac-QDs with different lactose densities and a PEGylated ($n = 3$) lactose-QDs conjugate (LacPEG-QDs) with more flexible sugar ligands were prepared. The amount of the sugar molecules on QDs can be determined by NMR, which was in agreement with the results from TGA determination. The formula of the conjugates was determined with ICP-OES. The interactions between the conjugated QDs and the PNA protein were measured using SPR, which revealed that higher lactose density favored binding affinity under the same concentration, and Lac-QDs exhibit higher affinity than LacPEG-QDs. We further used a solid phase assay to assess the anti-adhesion activity of Lac-QDs and LacPEG-QDs on the cell level. The results showed that Lac-QDs had stronger activity in preventing THP1 from adhering to HUVEC than LacPEG-QDs, which was consistent with the SPR results. We reasoned that decrease in the conformational entropy induced by appropriate restriction of sugar flexibility could enhance the binding affinity of glyco-QDs, which implies that entropy change may be the main contributor to the interaction between high valent glyco-QDs and protein. The fabrication of lactose on QDs provides a fluorescent multivalent carbohydrate probe that can be used as mimics of glycoprotein for the study of carbohydrate–protein interactions and cell imaging.

1. Introduction

Carbohydrate–protein interactions are important in a wide variety of physiological and pathological processes, such as cell-adhesion, cellular signalling, and inflammation, as well as infections induced by viral and bacterial agents.[1–4] There are several lines of evidences showing that typical monomeric carbohydrate–protein interactions are weak and nonspecific.[5] Carbohydrate–protein binding interactions have dissociation constants in the mM range and different carbohydrate ligands have similar affinities for the same protein receptor. Cells overcome this paradox to mediate specific cellular processes by the strategy of polyvalency, namely 'cluster-glycoside effect'.[6] It is believed that protein receptors that produce highly specific cellular responses contain clustered multiple carbohydrate-binding sites. Because of the complexity of this interaction, simplified model systems are usually used to study multivalent carbohydrate–protein interactions. In recent years, a number of spherical or linear arrays of oligosaccharides have been designed for mimicking the multivalent displaying of oligosaccharide ligands and studying the carbohydrates involved in bio-recognitions in natural process.[7–11]

Due to the similar sizes of nanoparticles (NPs) and most glycoproteins, the oligosaccharide ligands assembled on the NPs could achieve the specific geometry and orientation that the sugar moieties display on the surface of glycoproteins. Also, in this nanotechnology method, NPs can be used as the vector or probe for biorecognition studies, considering their specific physical properties.[12] It is known that quantum dots (QDs) exhibit unique optical properties, such as broad absorption, sharp luminescent emission, high quantum efficiency, and high photostability. Taking advantage of QDs to template oligosaccharide clustering assemblies has been implemented by some groups in recent years, which offers an opportunity for developing fluorescent carbohydrate biolabels.[13–18]

Previous studies demonstrated that the interaction between leukocytes and endothelial cells plays an important role in the patho-

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genesis of shock.[19] Some glycosyl moieties are directly involved in the interactions between adhesion molecules expressed on the surface of leukocytes and endothelial cells. We have demonstrated that tetra- and divalent lactose derivatives Gu-4, **6** and An-2, **7** (Fig. 1) function as antagonists against CD11b on leukocytes. They inhibit the adhesion of leukocytes to microvessel endothelial cells during shock, while monovalent lactose does not.[20]

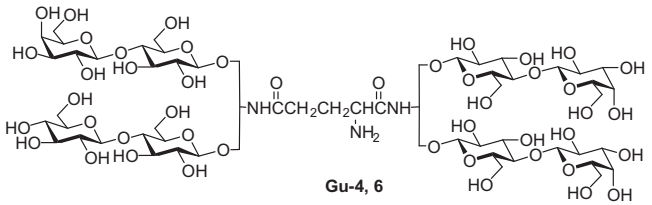
Based on these findings, we designed and fabricated the polyvalent lactosyl QDs (Lac-QDs) for specific labeling of live leukocytes. We also studied the cytotoxicity and photostabilities of Lac-QDs against a wide pH range (4.0–9.0) and reductive glutathione (GSH). The results showed that the Lac-QDs display good colloidal stability and low cytotoxicity.[21] In this paper, we further prepared a series of Lac-QDs with different lactose densities and a PEGylated (n = 3) lactose-QDs conjugate (LacPEG-QDs). With Nuclear Magnetism Resonance (NMR), Thermogravimetric Analysis (TGA) and Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), we analyzed the structure of the QDs and glyco-QDs. Carbohydrate–protein interactions were investigated using Surface Plasmon Resonance (SPR) and Solid Phase Assay. Finally we showed that Lac-QDs with no linker have the highest binding affinity in carbohydrate–protein interactions. The glyco-QDs could be applied as a fluorescent probe in cell imaging and carbohydrate–protein interaction study.

2. Results and discussion

2.1. Design and synthesis of the glyco-QDs

Ternary core/shell CdSeS/ZnS QDs was employed as the nanoscale platform to construct the multivalent lactosyl QDs. Unlike duplicate QDs (CdSe, CdS, CdTe), the emission wavelength of ternary QDs could be adjusted by the amount of Se, without changing the size of QDs.[22,23] Orange (λ_{em} = 564 nm) and green (λ_{em} = 506 nm) QDs were synthesized and analyzed by ICP-OES. The results showed that the orange QDs have a higher percentage of Se (11%) than the green one (0.8%, SI). Transmission Electron Microscopy (TEM) revealed that the two QDs have a diameter of 4.5 ± 0.5 nm (SI).

After preparing the QDs, the sugar compound was synthesized to modify QDs surface. However, there are still some difficulties in the preparation of the sugar compound, which present multiple hydroxyl groups requiring selective protection. Thiols groups were then introduced to the sugars for selective binding with QDs. Several research groups have developed different strategies to prepare glyco-QDs, but the synthetic procedure needs multi-step synthesis, protection, deprotection, and purification by chromatography.[13–15,18]



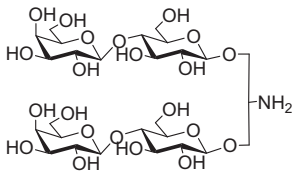


Figure 1. Gu-4, **6** and An-2, **7** which function as inhibitors against leukocyte adhesion.

Some 'one-pot' glyco-QDs synthetic methods were tried by opening the reducing end of the sugar unit.[16,24] To overcome these challenge, we developed a facile and convenient method for preparation of biocompatible Lac-QDs **1**, in which 1-thiol lactose (LacSH) was directly attached to QDs.[21] LacSH was synthesized in three steps from lactose and no step required purification.[25] PEGylated (n = 3) lactose (LacPEGSH) that was widely used in glycosylation of QDs was also prepared according to the previous reports (Scheme 1).[26]

It is known that the expression pattern of cell-surface carbohydrates changes during development and differentiation.[27] Carbohydrate surface density is used as an 'on–off' switch in the regulation of biological events.[28] Preparation of glyco-QDs with variable sugar density would be useful in glycobiology research. In this paper, a series of glyco-QDs with different lactose densities (**3**–**5**) were prepared by changing the ratio of LacSH and EtSH (1:1, 1:5, 1:10) (Scheme 1). Phase transfer reaction system was used in the functionalization of QDs. The reaction progress can be conveniently monitored with a UV lamp, as the luminescent QDs were transferred from the chloroform phase in the bottom to the upper PBS layer (SI) (Scheme 2).

2.2. Characterization of the QDs and glyco-QDs

The characterization of the structure and composition of glyco-QDs is also difficult, especially the determination of the number of sugars on QDs. Traditionally the number of carbohydrate on QDs is chemically analyzed by phenol-sulfuric acid method.[15–17] However, the procedure is complex and the result is strongly affected by the heating time and the duration of the experiment. Another disadvantage is that the method cannot differentiate various types of carbohydrates and other molecules. To quantitatively determine the number of sugar molecules on QDs surface, we developed a facile strategy using NMR that can provide more structural information and does not destroy the glyco-QDs. In the experiments, methanol and acetonitrile were used as internal standards (IS), which display NMR signals different from QDs and glyco-QDs. By peak integration, the amount of the ligands could be quantitatively obtained. We further demonstrated that the results from NMR were consistent with the results by Thermogravimetric Analysis (TGA) (Table 1).

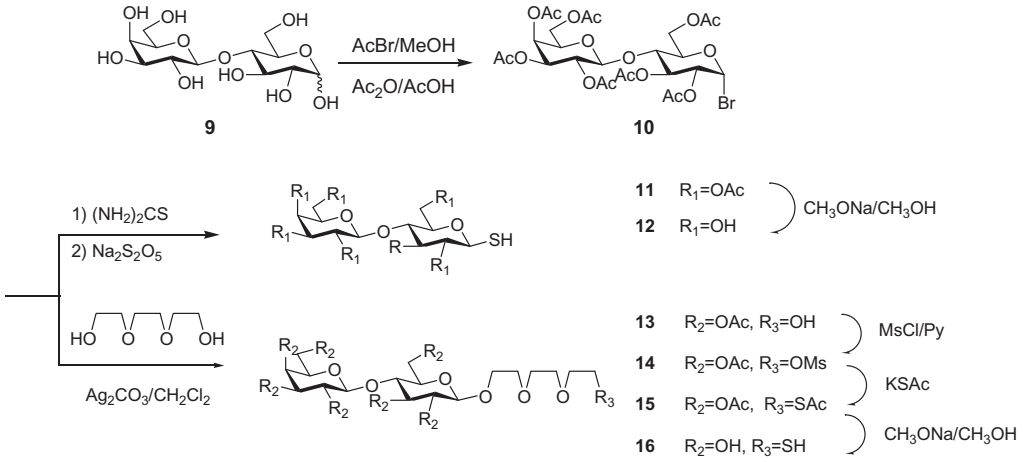
We used ^{1}H NMR to characterize QDs. ^{1}H NMR data of the QDs showed a characteristic signal of OA alkene hydrogen at 5.33 ppm (Fig. 2). Based on the integration of ^{1}H NMR, a molar ratio of TOPO to OA can also be obtained.

^{1}H NMR of QDs_{506} (400 M, 5 mg dissolved in $CDCl_3$, 1 μL methanol as IS): δ 0.88–2.00 (m, 48.36H); 3.49 (s, 16.44H, $-CH_3$ in CH_3OH); 5.31 (br, 2H). According to the results above we can calculate the molar ratio of TOPO:OA = 7:31. The amount of the TOPO and OA was 1.65 mg, weighing a percentage of 33.1%.

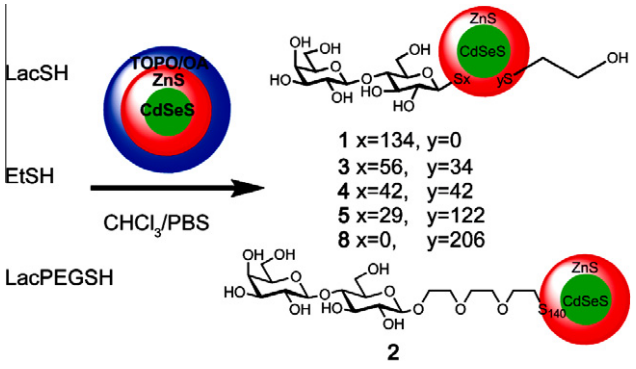
Then NMR was used to determine the amount of LacS on the QDs. ^{1}H NMR of **1** shows two anomeric hydrogen peaks of lactose at 4.35 ppm (q, $J_{1,2}$ = 7.6 Hz) and 4.52 ppm (q, $J_{1,2}$ = 9.2 Hz) (Fig. 3). Sometimes the anomeric hydrogen became quadruplicate peaks, likely because of the instability of nanocrystals coated with hydrophilic thiols[29–31] or microheterogeneity. By peak integration, we calculated that about 3.56 μmol of LacSH has been loaded onto the QDs surface, corresponding to 31.8% of total weight.

^{1}H NMR of LacPEG-QDs, **2** (300 M, 5 mg in D_2O with 1 μL acetonitrile as IS): δ 1.90 (s, 15.64H, $-CH_3$ in CH_3CN); 3.15–3.89 (m, 24H); 4.28 (d, 1H, J = 7.8 Hz, H-1′); 4.36 (d, 1H, J = 7.8 Hz, H-1). LacPEGS was calculated at 44.7% of total weight.

Together with the elemental contents obtained by ICP-OES, and the sizes of the QDs and LacSH calculated with the density functional theory (DFT) method, we further calculated the formula of **1** as $[Cd_{663}Se_{16}S_{644}–(ZnS)_{17}]@(LacS)_{134}$. Similarly, **2** was deter-



Scheme 1. Sythesis LacSH and LacPEGSH.



Scheme 2. Coating of the quantum dots.

Table 1
The percentages of the ligand weight on the surface of QDs characterized by NMR and TGA

Entry	NMR	TGA	Calcd
QDs (λ_{em} = 506 nm)	33.1	31.3	31.1
QDs (λ_{em} = 564 nm)	26.6	23.9	25.4
1	31.8	30.5 (39.23[a])	32.6
2	44.7	40.2 (40.95[a])	40.9
3	12.1	14.1	18.6
4	13.0	12.1	15.5
5	12.6	12.3	16.7
8	12.7	14.3	13.9

[a] Quantitive analysis by anthrone sulfuric acid assay. This method was only suitable for sugar coated QDs **1** and **2**, which could not be used to determine the whole ligand weight on other QDs.

mined as $[Cd_{663}Se_{16}S_{644}–(ZnS)_{17}]@(LacPEGS)_{140}$ in agreement with the previous reports for the carbohydrate-encapsulated 5 nm diameter QDs[17] and 6 nm gold-nanoparticles.[32] The formula of EtS-QDs, **8** was determined as $[Cd_{663}Se_{16}S_{644}–(ZnS)_{17}]@(EtS)_{206}$.

Moreover, the integration of ^{1}H NMR can also be used to determine the molar ratio of LacSH and EtSH. With acetonitrile as the IS, $QDs@(LacS)_{56}(EtS)_{34}$ **3**, $QDs@(LacS)_{42}(EtS)_{42}$ **4** and $QDs@(LacS)_{29}(EtS)_{122}$ **5** can be determined.

2.3. Bio-affinity study of the glyco-QDs

Generally, oligosaccharide was anchored onto QDs with a flexible thiol-linker [usually PEG (n = 3)] to improve accessibility as in

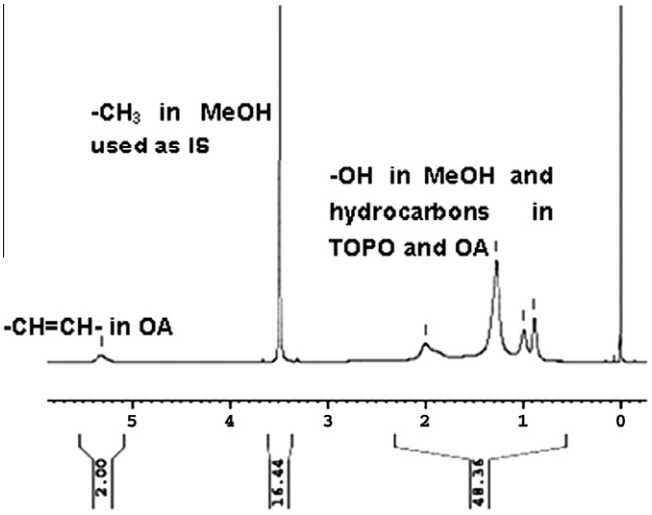


Figure 2. ^{1}H NMR spectrum of QDs (λ_{em} = 506 nm) with methanol as the internal standard.

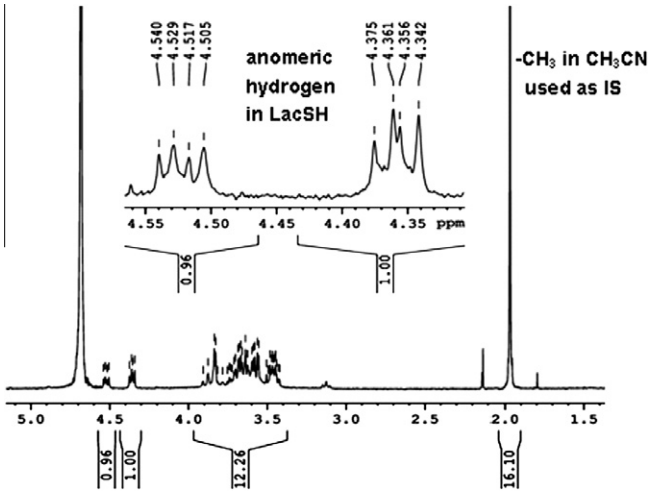


Figure 3. ^{1}H NMR spectrum of **1** with acetonitrile as internal standard.

2.[33] In our fabrication no linker was used. To determine if the linker affects the properties of the glyco-QDs, we investigated the

interaction between various lactose glyco-QDs and PNA protein by SPR. PNA is a lectin from *Arachis hypogaea* (Peanut), and it can specifically bind oligosaccharides bearing a terminal galactose moiety.[34] Two lactosyl derivatives, An-2, **7** and Gu-4, **6** were synthesized and tested as bivalent and tetravalent ligands, respectively. Figure 4 reveals that the binding curve of **1** is similar to that of **2**, indicating **1** retains its lectin specificity. It is also obvious that multivalent presentation is important in achieving high affinity in carbohydrate interactions.[35] Although the concentrations of **1**–**5** were much lower than that of An-2 and Gu-4, they showed a stronger SPR binding signal indicating more material is retained on the sensor surface. As expected, **8** showed no binding activity, indicating the interaction with the carbohydrate is highly specific and the strong affinity was not due to the QDs particles (Table 2).

Although we calculated the formulas of the glyco-QDs from ICP-OES, NMR and TGA results, it is only a prediction and the precise number of ligand-target interactions is also not known. So the molar concentration, k_d and k_a values could not be accurately measured from SPR. However, from the curve of SPR, we can analyze the kinetic properties of the interaction. Figure 4 shows that **1** and **2** have slower association and dissociation rates in the interaction with PNA. But small molecules **6**, **7** and QDs with lower density of the lactose (**3**, **4**, **5**) showed faster association and disassociation rates. It has been suggested that cell-surface carbohydrate density regulates biological events. Our finding may suggest that at low sugar density, carbohydrate ligands are not close enough to permit multivalent binding. In contrast, tight protein binding occurs when the surface density of the particular carbohydrate ligand is higher.[27]

Interestingly, even though the number of lactose on **1** (134 sugar molecules on each quantum dots) is similar to that on **2** (140 sugar molecules on each quantum dots), in which the lactose was attached to the QDs with a PEG ($n = 3$) flexible linker, **1** has stronger binding affinity to PNA than **2**. Apparently, valence is not the only main factor in the glyco-QDs protein interactions. There are possibly other important factors influencing the interaction.

Our observation suggested that appropriate restriction of sugar flexibility may enhance the binding affinity of glyco-QDs. The conformational entropy[6] could become the main factor in high valent glyco-QDs. Similar observation has been reported by Wu and Chen et al. on mannose capped gold-nanoparticles,[36] that the shorter linker chain resulted in higher binding affinity to Con A (lectin from *Concanavalin A*, binding specifically to manno- and glucopyranosides[5]). We note that this phenomenon is also reported by Russell et al. on lactose stabilized gold-nanoparticles mimicking carbohydrate–carbohydrate interactions.[26]

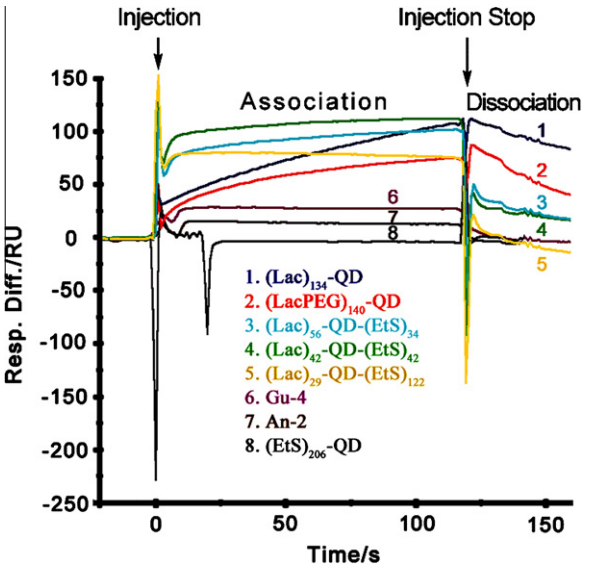


Figure 4. SPR study of the lectin binding of glyco-QDs.

Table 2
The concentration and RU value of **1–8**

Entry	Concentration[a] (mM)	RU[b]
1	6×10^{-3}	103.42
2	6×10^{-3}	81.08
3	8×10^{-3}	47.21
4	8×10^{-3}	39.79
5	8×10^{-3}	21.13
6	0.5	7.42
7	0.5	1.62
8	8×10^{-3}	0

[a] Approximate concentration of glyco-QDs calculated by predicted formulas.
[b] RU is the highest response signal after injection stopped.

2.4. Anti-adhesion study of the glyco-QDs

We further used a solid phase assay to assess the anti-adhesion activity of **1** and **2** to prevent THP1 from adhering to HUVEC. An-2, Gu-4 and two anti-human CD11b antibodies, ICRF44 (anti-human

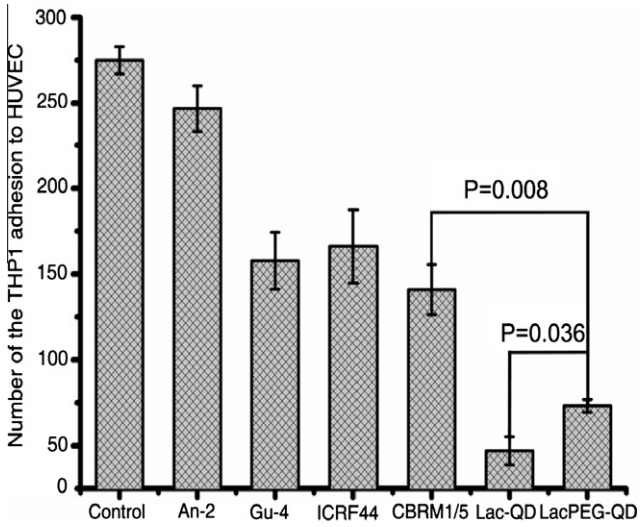


Figure 5. Leukocyte-endothelial cell-adhesion assay. An-2 and Gu-4 were 40 nmol/ml; ICRF44 and CBRM1/5 were 40 ng/ml; **1** and **2** were 0.4 nmol/ml. Statistical analysis of THP1 adhesion to HUVEC was performed by counting 5 microscopic fields of vision for each lactose derivative. Data are presented as mean ± SEM for each group.

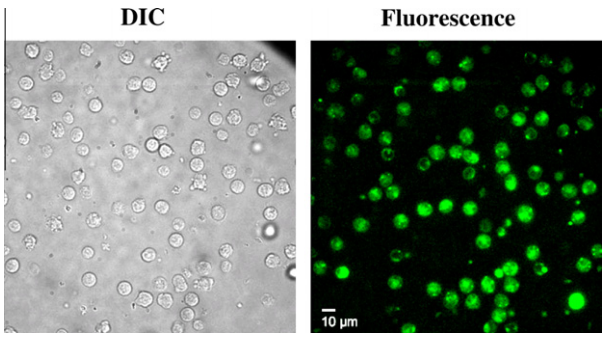


Figure 6. Lac-QDs used for the fluorescent labelling of live leukocytes.

CD11b, Clone: ICRF44) and CBRM1/5[37] were used as positive controls (Fig. 5). The results indicated that the two glyco-QDs have the strongest anti-adhesion activity, even though their concentrations are the lowest. The results also showed that **1** has stronger binding affinity to THP 1 than **2**, which is consistent with the SPR results. Compared with **2**, the synthesis of **1** is easier and it has better activity, so we finally chose **1** as a fluorescent probe to image live leukocytes.

2.5. Cytotoxicity measurement and leukocyte labelling

The fluorescence emission spectrum of **1** was basically the same as that of QDs (SI). We used **1** as a fluorescent probe and showed that the lactoses on modified QDs binds to leukocyte cell surface (Fig. 6). Cd containing compounds are harmful to living cells,[38] but the ZnS shell protects cells from Cd exposure. Our toxicology experiment suggested that **1** and **2** were not toxic to Hela cells (cancer cell lines of human origin) under a concentration of 0.5 mg/ml[21] and HUVEC (normal cell lines of human origin) under a concentration of 50 μg/ml (Fig. 7).

3. Conclusion

We have developed a convenient approach to regulate the density of the lactose on QDs by adding another thiol ligand 2-mercaptoethanol and a new strategy to determinate the amount of lactose on the QDs using NMR, which has several advantages over traditional methods. The NMR results are in good agreement with that obtained by TGA.

The biological assay showed that oligosaccharides coated on QDs surface can dramatically enhance their binding activity through cluster effect. The results also demonstrated that a functional assembly of lactose on QDs surfaces could be achieved using 1-thiol substituted lactose without a spacer. Our SPR study showed how changes in the composition and the density of cell-surface carbohydrates influence biological recognition processes.

This work also clearly highlighted the importance of the length of linkers that anchor carbohydrates to the QDs surface and demonstrated an approach to design multivalent glycoclusters with high affinity. Glyco-QDs can be used as an efficient fluorescent probe to assess the carbohydrate–protein interactions and image the glycoproteins and their cellular activities. We hope the control of glyco-QDs composition and the characterization strategy will allow the design and fabrication of multifunctional glyco-QDs that can be used in targeted drug delivery.

4. Experimental

4.1. General methods

All starting materials, reagents and solvents were obtained from commercial suppliers and used as supplied without further purification. NMR spectra were recorded on a JEOL-300 (300 MHz) instrument or a Bruker AMX-400 (400 MHz) instrument. Thermo gravimetric analysis (TGA) was carried out on a Q50SDT TA instrument and the samples were burned in nitrogen at a constant heating rate of 10 °C/min from 25 to 950 °C. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was recorded on Thermo-iCAP 6000. Olympus IX 71 IX2 fluorescent microscope equipped with an SERIES EMCCD ixon DV887 (Andor) was used to study the fluorescent labeling of live leukocytes with Lac-QDs. Surface Plasmon Resonance (SPR) measurements were carried out with BIAcore 3000 instrument using carboxymethylated sensorchips (CM5, BIAcore). Leukocyte was separated from mouse blood. PNA lectin from *A.* hypogaea (Peanut) was purchased from Sigma–Aldrich (L0881). THP1 (Human acute monocytic leukemia cell line) was obtained from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, PR. China), HUVEC (Human Umbilical Vein Endothelial Cells) was purchased from ATCC. Calcein-AM was purchased from Sigma–Aldrich (C1359). Cell culture and antibody were obtained from commercial suppliers RPMI1640 (GIBCO 31800-022), ECM (ScienCell, Cat: 1001, Lot: 4158), anti-human CD11b (Biolegend 301312), CBRM1/5 (eBioscience 12-0113).

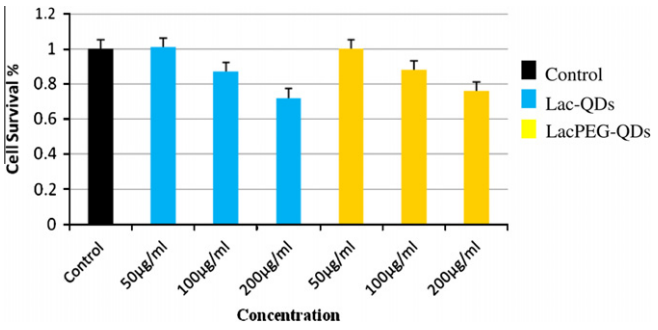


Figure 7. Cytotoxicities of Lac-QDs and LacPEG-QDs.

4.1.1. Synthesis of TOPO and OA stabilized CdSeS–ZnS QDs

Ternary core/shell CdSeS/ZnS QDs passivated with trioctylphosphine oxide (TOPO) and oleic acid (OA) were prepared according to the reported method with minor adjust of Se.[21,23] CdO (1.28 g), oleic acid (0.46 g), and 15 mL tri-*n*-octylamine were mixed in a three-necked flask and heated to 300 °C under inert atmosphere. A solution of Se (2.1 mg) and S (12.4 mg) in 1.0 mL tri-*n*-octylphosphine (TOP) was rapidly injected into the hot solution and the reaction was allowed to proceed for 1 min. ZnS stock solution (0.2 mM zinc oxide powder in 1.0 ml OA and 0.2 mM sulfur powder in 1.0 ml TOP) was then added dropwise (one drop per s) to the reaction solution at 220–240 °C. Green QDs (λ_{em} = 506 nm) were obtained with ethanol sedimentation and washed with ethanol for three times. The product is a yellow power.

Orange QDs (λ_{em} = 564 nm) were synthesized with the same procedure except for changing the ratio of the CdO (0.26 g), oleic acid (0.14 g) Se (12 mg) and S (192 mg). The product is a red power.

The green QDs was chosen for further research.

4.1.2. 2,3,6,2,3,4,6-Sept-*O*-acetyl-α-D-lactosyl bromide (10)

Acetyl bromide (0.92 mL, 12.3 mmol) and CH_3OH (0.5 mL, 12.30 mmol) were added to AcOH (10 mL) and stirred at rt (protected from light) for 15 min, followed by addition of lactose (1.0 g, 3.08 mmol) and Ac_2O (3.4 mL, 35.97 mmol). The reaction mixture was further stirred overnight at rt and the solution was diluted with chloroform, washed with saturated solution of sodium bicarbonate and dried with sodium sulfate. The solvent was removed, and ether was added to give title compound **10** (2.15 g, yield: 95%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97–2.18 (m, 21H, 7 × OAc); 3.84–3.93 (m, 2H); 4.05–4.22 (m, 4H); 4.49–4.53 (m, 2H); 4.77 (dd, 1H, J = 3.9, 9.9 Hz, H-2); 4.97 (dd, 1H, J = 3.3, 10.5 Hz, H-3); 5.13 (dd, 1H, J = 7.8, 10.2 Hz, H-2); 5.35 (d, 1H, J = 3.3 Hz, H-4); 5.56 (t, 1H, J = 9.9 Hz); 6.53 (d, 1H, J = 3.9 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.40, 20.56, 20.70, 60.78, 60.93, 66.50, 68.89, 69.46, 70.66, 70.72, 70.88, 72.87, 74.85, 86.33, 100.73, 168.86, 169.13, 169.88, 169.97, 170.05, 170.24; mp 138–142 °C.

4.1.3. 2,3,6,2,3,4,6-Sept-*O*-acetyl-1-thiol-β-D-lactose (11)

A mixture of **10** (2.5 g, 3.58 mmol) and thiocarbamide (1.63 g, 21.46 mmol) in acetone (20 ml) was refluxed for 0.5 h, After cooling and concentrating, a solution of sodium pyrosulfite (1.36 g) in water (20 ml) was heated at 85 °C and chloroform (30 ml) was introduced, followed by addition of the concentrated reaction solu-

tion. The reaction mixture was boiled for 15 min with constant stirring. After cooling, the organic layer was separated, washed with water, and dried with sodium sulfate. The solution was filtered and evaporated under diminished pressure to give a crude syrup **11**. (2.15 g, yield: 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97–2.18 (m, 21H, 7 × OAc); 2.27 (d, 1H, J = 9.6 Hz, -SH); 3.62–3.66 (m, 1H);3.78–3.91 (m, 2H); 4.05–4.16 (m, 3H); 4.44–4.57 (m, 3H); 4.88 (t, 1H, J = 9.9 Hz,); 4.96 (dd, 1H, J = 3.9, 10.5 Hz, H-3); 5.10 (dd, 1H, J = 7.8, 10.2 Hz, H-2); 5.18 (t, 1H, J = 9.3 Hz); 5.35 (d, 1H, J = 3 Hz, H-4); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.47, 20.61, 20.73, 20.85, 60.73, 62.15, 66.50, 68.96, 70.63, 70.91, 73.39, 73.78, 76.01, 76.58, 78.40, 101.06, 169.04, 169.57, 170.09, 170.32; ESI-TOF calcd for $C_{26}H_{36}O_{17}SNH_4^+$ ($M + NH_4^+$) 670.21, found 670.21; calcd for $C_{26}H_{36}O_{17}SNa^+$ (M+Na$^+$) 675.17, found 675.17.

4.1.4. 1-Thiol-β-D-lactose (12)

A solution of crude compound 11 (1.35 g, 5.13 mmol) in MeOH (10 mL) was treated with a solution of sodium in MeOH (1 mL). After 1 h, the solution was acidified with DOWEX 50WX4-100 ion-exchange resin (Sigma–Aldrich) to pH 7.0. Evaporation of the solution under reduced pressure gives white solid foam **13** (0.7 g, yield: 95%). ^{1}H NMR (300 MHz, D_2O): δ 3.33–3.79 (m, 12H); 4.27 (d, 1H, J = 7.5 Hz, H-1); 4.403(d, 1H, J = 9.6 Hz, H-1); ^{13}C NMR (75 MHz, D_2O): δ 60.58, 61.58, 69.08, 71.47, 73.02, 78.49, 79.51, 80.45, 103.38; ESI-TOF calcd for $C_{12}H_{22}O_{10}SNa^+$ (M+Na$^+$) 358.09, found 358.09.

4.1.5. (8-Hydroxy-3,6-dioxaoctyl)2,3,6,2,3,4,6-hepta-O-acetyl-β-D-lactoside (13)

2,3,6,2,3,4,6-Sept-*O*-acetyl-α-D-lactosyl bromide, **10** (15 g, 21.5 mmol) and triethyleneglycol (26 ml, 193.1 mmol) was dissolved in dry dichloromethane (200 ml), stirred at room temperature with activated molecular sieve (4 Å) under an inert atmosphere for 30 min. Then silver carbonate (8.5 g, 30.8 mmol) was added and the reaction mixture was stirred at room temperature in the dark for 24 h. The mixture was filtered and washed with dichloromethane. The solution was washed with cool water and dried with sodium sulfate. Chromatographic purification gives the title compound **13** (11.4 g, 69.2%) as a syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.9–2.15 (m, 21H, 7 × OAc); 2.64 (br, 1H, –OH); 3.58–3.66 (m, 9H, –OCH_2 in PEG); 3.71–3.74 (m, 3H); 3.79(t, 1H, J = 9.6, 9.2 Hz); 3.88–3.92 (m, 2H); 4.08–4.14 (m, 3H); 4.47–4.51(m, 2H); 4.61 (d, 1H, J = 8 Hz,); 4.89 (dd, 1H, J = 8, 9.2 Hz); 4.96 (dd, 1H, J = 3.2, 10.4 Hz); 5.11 (dd, 1H, J = 8, 10.4 Hz); 5.19 (t, 1H, J = 9.2, 9.2 Hz); 5.34 (d, 1H, J = 2.8 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.33, 20.45, 20.54, 20.64, 20.68, 60.73, 61.52, 61.90, 66.56, 68.76, 69.03, 70.19, 70.22, 70.54, 70.84, 71.56, 72.41, 72.50, 72.68, 100.39, 100.87, 168.97, 169.56, 169.63, 169.87, 169.99, 170.18, 170.24.

4.1.6. (8-Acetylthio-3,6-dioxaoctyl)2,3,6,2,3,4,6-hepta-*O*-acetyl-β-D-lactoside (15)

To a solution of alcohol **13** (0.4 g, 0.52 mmol) in pyridine (5 mL) was added methanesulfonyl chloride (0.1 mL. 0.148 g, 1.3 mmol), stirred at room temperature for 1 h, CH_2Cl_2 (25 mL) was added and the organic solution was washed sequentially with 1 M hydrochloric acid (100 mL), $NaHCO_3$ (aq) (50 mL), and water (50 mL) and then dried with Na_2SO_4. TLC indicated complete conversion of the starting alcohol into the methanesulfonate **14** (0.42 g, 85%), which was used directly in the next step. A mixture of the methanesulfonate **14** (0.38 g, 0.63 mmol) and potassium thioacetate (0.144 g, 1.26 mmol) in butanone (25 mL) was heated under reflux for 2 h, when TLC showed that the starting material had been replaced with the thioacetate. The solvent was removed under reduced pressure, and the residue was distributed between CH_2Cl_2 (25 mL) and water. The separated organic layer was dried and concentrated, and the product was subjected to column chromatography (EtOAc–Petrol ether, 1:1) to give thioacetate **15** (0.26 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97–2.34 (m, 24H, 8 × OAc); 3.09 (t, 2H, J = 6.8 Hz, –CH_2 in –CH_2SAc); 3.57–3.64 (m, 8H, –OCH_2 in PEG); 3.69–3.74 (m, 1H); 3.79 (t, 1H, J = 9.9, 9.2 Hz); 3.86–3.92 (m, 2H); 4.05–4.15 (m, 3H); 4.48–4.58 (m, 3H); 4.89 (dd, 1H, J = 8, 9.2 Hz); 4.94 (dd, 1H, J = 3.2, 10.4 Hz); 5.10 (dd, 1H, J = 8, 10.4 Hz); 5.19 (t, 1H, J = 9.2, 9.2 Hz); 5.10 (d, 1H, J = 2.8 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.42, 20.55, 20.64, 20.73, 20.79, 28.71, 29.61, 30.49, 60.74, 61.97, 66.56, 69.02, 69.05, 69.72, 70.25, 70.55, 70.61, 70.93, 71.61, 72.56, 72.76, 76.23, 100.56, 101.01, 168.98, 169.57, 169.67, 169.96, 170.06, 170.25, 170.28, 195.41.

4.1.7. (8-Thiol-3,6-dioxaoctyl)-β-D-lactoside (16)

A solution of **15** (1 equiv) in dry MeOH (25 mL) was treated with 0.05 M methanolic solution of NaOMe. The reaction was stirred under an argon atmosphere. After 1 h, the solution was acidified with DOWEX 50WX4–100 ion-exchange resin (Sigma–Aldrich) to pH 7.0. Evaporation of the solution under reduced pressure gives a syrup. ^{1}H NMR (400 MHz, D_2O): δ 2.87 (t, 2H, J = 6.4 Hz, –CH_2 in –CH_2SH); 3.36–3.93 (m, 20H); 4.29 (d, 1H, J = 7.6 Hz); 4.36 (d, 1H, J = 8 Hz); ^{13}C NMR (100 MHz, D_2O): δ 37.18, 60.00, 60.04, 60.97, 68.28, 68.34, 68.50, 68.70, 69.29, 69.49, 69.64, 70.90, 72.46, 72.77, 74.26, 74.72, 75.38, 78.33, 102.04, 10.89.

4.1.8. Surface capping of CdSe–ZnS QDs with LacSH, LacPEGSH and EtSH

The exchange of TOPO/OA capped QDs with LacSH, 2-mercaptoethanol (EtSH) and LacPEGSH were carried out by phase transfer reaction between chloroform phase and aqueous phase of phosphate buffered saline (PBS). Generally, A solution of QDs_{506} (10 mg) in $CHCl_3$ (10 mL), and thiol compounds (100 mg) in PBS (30 ml) was stirred at room temperature for 4 h. When the lower layer is colorless and the upper becomes yellow, it indicates the reaction was finished. After standing for a while, two layers separated naturally. The upper aqueous layer was collected and purified with Sephdex G-70 then lyophilized to give a yellow powder. Moreover, by coating with different molar ratios of EtSH and LacSH (1:1, 5:1 and 10:1), we can control the density of lactose on QDs.

4.2. Surface plasmon resonance analysis

The SPR results were obtained on the BIAcoreTM 3000 instrument (BIAcore, Uppsala, Sweden). Lectin-functionalized BIAcore sensor chips were prepared from carboxymethylated sensor chips (CM5, BIAcore) by NHS/EDC activation followed by injection of PNA in acetate buffer (10 mM, pH 4.5). PNA was immobilized on CM5 sensor chip at 4000 response unit (RU). Solutions (20 μL) of **1–8** in buffer were flown over the sensor chip with a rate of 10 μL/min. The data were analyzed with BIAevaluation 4.0 software.

4.3. Cytotoxicity testing results from MTT assay

HUVEC (Human Umbilical Vein Endothelial Cells) was trypsinized and re-suspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were seeded at a density of 0.2–1 million cells/well in a 96-well plate at 37 °C and 5% CO_2 for 24 h, and washed with PBS. Lac-QDs samples of desired concentrations (50, 100, 200 μg/mL) were added to each well with 6 duplicates for each concentration. After 24 h of incubation, the supernatant was removed, and the cells were washed with PBS three times. To evaluate cell viability, 100 μL of MTT solution were added to each well,

and incubated at 37 °C for 4 h. After incubation, each well was treated with 100 μL dimethylsulfoxide (DMSO) for 3–5 min. The optical absorbance was measured at 460 nm on a plate reader. Each data point was the average of 6 wells, and 100% viability was determined from the untreated cells.

4.4. Solid phase assay

HUVECs were cultured in 96-well microtiter plate. The fluorescent Calcein-AM labeled THP1 was first treated with lipopolysaccharide (LPS) followed by coated QDs, free polysaccharides, and CD11b antibodies (ICRF44 and CBRM1/5) for 10 min. At last, the treated THP1 was added into the wells containing HUVECs. The plate was incubated at 37 °C for 40 min, and the unbound THP1 cells were washed away. The plate was observed under a fluorescence microscope and the number of the leukocytes adhered to the endothelial cells were counted.

4.5. Fluorescent labeling study using a fluorescence microscope

Leukocytes from rats were isolated. After washing for three times with ice cold PBS and centrifuging at 400*g* for 5 min, cells were re-suspended with 1 ml of DMEM. Cell suspension was added to culture dishes and incubated at 37 °C, under 5% CO_2 for 1 h. Lac-QDs was diluted to a concentration of 1 mg/ml with PBS and added to Petri dishes (20 μL/dish). The dishes were kept at room temperature for 10 min and washed with PBS for three times to remove nonconjugated sugars. The images of the cells bound with Lac-QDs were observed under the fluorescence microscope.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.046.

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