

Cytosensing and dynamic monitoring of cell surface carbohydrate expression by electrochemiluminescence of quantum dots†

En Han, Lin Ding, Hongzhen Lian and Huangxian Ju*

Received 20th January 2010, Accepted 25th March 2010

First published as an Advance Article on the web 16th April 2010

DOI: 10.1039/c001331e

A novel electrochemiluminescent (ECL) cytosensing strategy for sensitive dynamic monitoring of carbohydrate expression on living cells was designed by combining the specific recognition of lectin to carbohydrate groups with the functionalization of immobilized CdSe quantum dots, which acted as ECL emitting species.

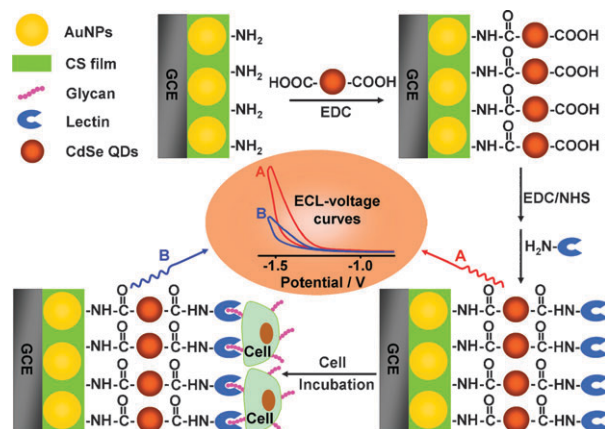
Carbohydrates, the key components of cell surface glycolipids and glycoproteins, play critical roles in numerous biological events including cell adhesion, signal transduction, immune responses, cell–cell communication, and tumor growth and metastasis.¹ Abnormal carbohydrate expression patterns are associated with many diseases, especially cancers.² Therefore, dynamic monitoring of carbohydrate expression on cell surfaces has become an important subject for understanding their roles in disease development and providing diagnostic tools to guide treatment.³

The analytical techniques for carbohydrate detection primarily include mass spectrometry, nuclear magnetic resonance, and chromatography.⁴ Although these techniques reveal molecular detail, they are unsuitable for analysis of carbohydrates on living cells because of their destructive nature. Although lectin arrays have been developed for analysis of cell surface carbohydrates by means of the highly specific binding of lectin to carbohydrate,⁵ the detection process generally involves the fluorescent labeling of cells,^{5a,b} leading to disturbance of the cellular nature and lack of sensitivity due to the autofluorescence of cells. Focusing on these problems, our previous works developed several methods for electrochemical monitoring of cell surface carbohydrate expression.⁶ Herein a novel strategy was designed to analyze cell membrane carbohydrates by combining the electrochemiluminescent (ECL) behavior of quantum dots (QDs) with the specific recognition of lectins to carbohydrates. This is the first work on the ECL cytosensing and dynamic monitoring of cell surface carbohydrate expression.

ECL technique possesses low cost, wide range of analytes, and high sensitivity,⁷ and the ECL behaviors of QDs in both organic⁸ and aqueous⁹ solutions have attracted considerable interest. In recent years the ECL phenomena of CdSe,^{9a} CdS^{9b} and CdTe QDs^{9c} have been used for the development of ECL analytical methods for detection of a series of chemical and biological species and the fabrication of immunosensor for

low-density lipoprotein^{9b} and enzyme-based ECL biosensor for glucose.^{9a} Here, the thioglycolic acid-capped CdSe QDs with a diameter of 1.8 nm (Fig. S1 in ESI†) were firstly covalently bound to chitosan (CS)-gold nanoparticles (AuNPs) composite modified electrodes and then further functionalized with four lectins individually (Scheme 1). These lectins included Concanavalin A (Con A), *Dolichos biforus* agglutinin (DBA), peanut agglutinin (PNA) and wheat germ agglutinin (WGA) (Table S1 in ESI†). The electrode modification and functionalization were characterized by atomic force microscopy and electrochemical impedance spectroscopy (Fig. S2 and S3 in ESI†). The resulting biofunctional films with four kinds of lectins showed excellent ECL behavior. Using human leukemic K562 cells as a model, the specific recognition of QD-bound lectins to cell surface carbohydrates could capture cells onto the electrode surface and thus decrease the ECL emission, which provided a simple and highly sensitive way for ECL cytosensing and dynamic monitoring of cell surface carbohydrate expression corresponding to the four lectins.

In a 0.1 M pH 7.4 phosphate buffered saline (PBS) containing 0.1 M K₂S₂O₈ and 0.1 M KCl, different CdSe QDs immobilized electrodes showed different ECL intensities at similar applying potentials (Fig. 1a). The ECL-voltage curves of bare glassy carbon electrode (GCE) and CS-AuNPs/GCE showed neglectable ECL emission, indicating the background ECL signal from the electrode without CdSe QDs were very low. The typical cyclic voltammogram of the QDs modified electrode in this system showed two cathodic peaks at -0.96 and -1.32 V (inset in Fig. 1a), which could be attributed to the reduction of S₂O₈²⁻ and CdSe QDs, respectively.^{9b} Upon immobilizing CdSe QDs on the same electrode, a strong ECL emission starting



Scheme 1 Schematic representation of ECL cytosensor for monitoring cell surface carbohydrate expression.

Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), Department of Chemistry, Nanjing University, Nanjing 210093, P.R. China. E-mail: hxju@nju.edu.cn; Tel: +86 25 83593593

† Electronic supplementary information (ESI) available: Experimental details, characterization of quantum dots and cytosensor fabrication. See DOI: 10.1039/c001331e

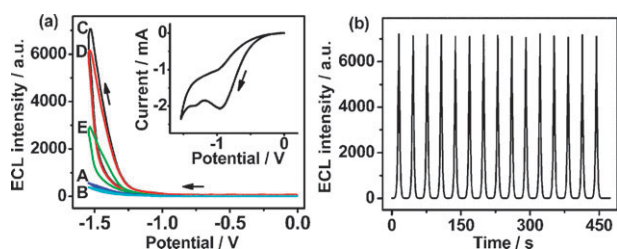


Fig. 1 (a) ECL-voltage curves of bare GCE (A), CS-AuNPs/GCE (B), QDs/CS-AuNPs/GCE (C), WGA/QDs/CS-AuNPs/GCE (D) and cells/WGA/QDs/CS-AuNPs/GCE (E), and (b) ECL emission from QDs/CS-AuNPs/GCE upon continuous 15-cyclic scans between 0 and -1.55 V in 0.1 M pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. Inset in (a): cyclic voltammogram of QDs/CS-AuNPs/GCE in the same solution. Scan rate: 100 $mV\ s^{-1}$. ECL emission comes from the excited state of QDs produced at electrode surface.⁷

at about -1.2 V showed a peak value at around -1.53 V (curve C, Fig. 1a). The mechanism was similar to the previous reports.^{9b,10} Briefly, when the potential was scanned with an initial negative direction, the CdSe QDs immobilized on the electrode were reduced to form the anion radical ($\bullet CdSe^-$) by electron injection, and the reduction of $S_2O_8^{2-}$ at -0.96 V produced a strong oxidant, $\bullet SO_4^-$. The $\bullet SO_4^-$ could react with $\bullet CdSe^-$ to produce an excited state of the QDs ($CdSe^*$) to emit an ECL signal. Specifically, the ECL emission of the QDs immobilized electrode was very stable. Upon successive scans between 0 and -1.55 V for 15 cycles, no obvious change was observed (Fig. 1b). The result suggested that the designed interface was suitable for ECL detection.

After WGA was covalently conjugated to the QDs/CS-AuNPs film, the ECL signal of the immobilized QDs slightly decreased (curve D, Fig. 1a), which was due to the increased electron-transfer resistance (Fig. S3 in ESI[†]) and the inhibition of the protein molecules to the reaction between $\bullet SO_4^-$ and $\bullet CdSe^-$. A great decrease of ECL signal was observed after the WGA/QDs/CS-AuNPs/GCE was incubated with K562 cell suspension for 1 h (curve E, Fig. 1a). The reason was that the binding of cells to the WGA on electrode through the specific recognition between cell surface carbohydrate and lectin greatly inhibited the electron transfer reactions on the electrode surface. Furthermore, the decrease in ECL emission intensity (ΔI_{ECL}) was related to the cell number captured on the electrode by lectin-carbohydrate binding. The latter depended on the incubation condition and the carbohydrate expression on cell surface, producing a method of cytosensing for detection of cell concentration in incubation solution and monitoring of cell surface carbohydrate expression.

The concentrations of four lectins for functionalizing the QDs/CS-AuNPs film were optimized, respectively (Fig. 2a). The ECL profiles of four lectin-immobilized electrodes after incubation with the same cell concentration were shown in inset in Fig. 2b. The decrease in magnitude of the ECL emission at the four electrodes upon incubation was in the order: $D > C > B > A$ (inset in Fig. 2b), which corresponded to the decreasing cell binding ability of these lectin-immobilized electrodes in the same order: $WGA > Con\ A > PNA > DBA$ (Fig. 2b). The results reflected the distinct expression extent of different carbohydrates on cell surfaces, and suggested high

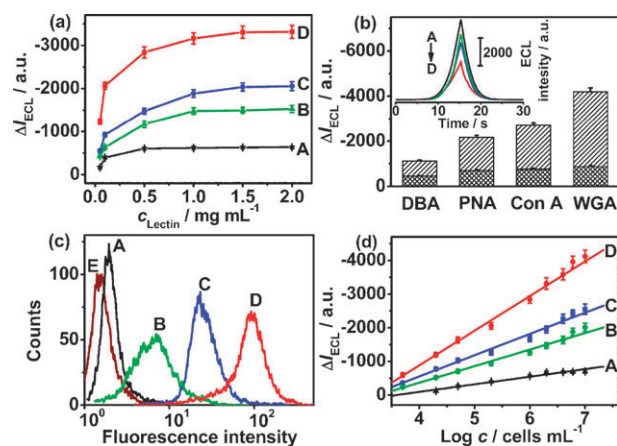


Fig. 2 (a) Plots of ΔI_{ECL} values at DBA (A), PNA (B), Con A (C) and WGA (D) immobilized electrodes upon incubation in 2.0×10^6 cells mL^{-1} K562 cell for 1 h vs. concentration of lectins; (b) ΔI_{ECL} values of lectin/QDs/CS-AuNPs/GCEs compared to QDs/CS-AuNPs/GCE (hash) and cells/lectin/QDs/CS-AuNPs/GCEs compared to lectin/QDs/CS-AuNPs/GCEs (stripes); (c) Flow cytometric analysis of FITC-DBA (A), FITC-PNA (B), FITC-ConA (C) and FITC-WGA (D) treated K562 cells and autofluorescence (E); and (d) plots of ΔI_{ECL} values at DBA (A), PNA (B), Con A (C) and WGA (D) immobilized electrodes vs. logarithm value of K562 cell concentration. Inset in (b): ECL profiles of DBA (A), PNA (B), Con A (C) and WGA (D) immobilized electrodes upon cell binding. ECL intensities in this work were the ECL peak values in 0.1 M pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$ at 100 $mV\ s^{-1}$.

expression of WGA-recognizing carbohydrate, moderate expression of mannose groups (Con A-recognizing carbohydrate), and low expression of PNA- and DBA-recognizing carbohydrates on K562 cell surfaces. The flow cytometric analysis using lectins labeled with fluorescein isothiocyanate (FITC) for recognition indicated the expression levels of cell membrane carbohydrates followed the order: $WGA > Con\ A > PNA > DBA$ (Fig. 2c), which was in good agreement with that monitored by the designed ECL method and previous reports.^{6a,11} Thus, the ECL strategy could accurately evaluate the carbohydrate expression pattern on cell surfaces.

The ΔI_{ECL} values obtained on four lectin/QDs/CS-AuNPs/GCEs upon incubation were proportional to the logarithmic value of cell concentration in the incubation solution (Fig. 2d). The detection limits of WGA, Con A, and PNA-modified electrodes for cell concentration were calculated to be 1.1×10^3 , 1.6×10^3 and 2.1×10^3 cells mL^{-1} , respectively, which were lower than that with the limit of 6×10^3 cells mL^{-1} at an immunosensor chip for detection of *E. coli* O157:H7.¹² The slope of plots varied for different lectins, which indicated different expression extents of these carbohydrates on K562 cell surface. The intra- and interassay imprecisions for detection of carbohydrates on cell surfaces with the designed ECL cytosensors were investigated by use of one cytosensor for four replicated measurements and four kinds of the designed cytosensors for one cell sample at the same time. The intraassay variation coefficients (CVs) were less than 4.7% and interassay CVs were less than 8.2% (Table S2 in ESI[†]), showing acceptable reproducibility.

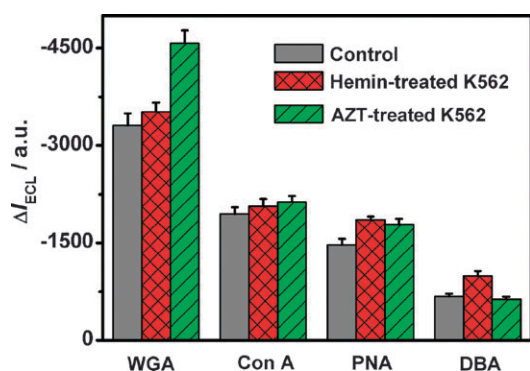


Fig. 3 ΔI_{ECL} values obtained at different lectin-immobilized electrodes after incubation in 2.0×10^6 cells mL^{-1} of K562 cells as control, hemin- and AZT-treated K562 cells for 1 h.

This strategy could easily be used to monitor the dynamic changes of cell surface carbohydrates in response to drugs. Hemin and 3'-azido-3'-deoxythymidine (AZT) were selected as two model drugs to demonstrate the practicality of this strategy (Fig. 3). Hemin can promote K562 cells to differentiate into erythroid lineages, resulting in an enhanced expression of erythroid marker such as glycophorin A.¹³ The intracellular metabolite of AZT can inhibit nucleotide-sugar transport and produces marked variations of the oligosaccharide moiety of glycoconjugates.¹¹

Compared with control cells, the hemin-treated K562 cells displayed greater ΔI_{ECL} values at PNA- and DBA-immobilized electrodes. Their increasing percentages were 26% and 47%, respectively. The WGA and Con A-immobilized electrodes did not give a statistically significant change of ECL emission upon incubation with hemin-treated K562 cell solution ($p > 0.05$, calculated by one-way ANOVA^{5b}). Thus after treatment with hemin, PNA and DBA-recognizing carbohydrates (*O*-glycans)^{14a} on erythroid differentiated K562 cell surface significantly increased. The results were in agreement with previous reports,^{14b,c} and could be explained by the increasing expression of glycophorin A, which contained 15 *O*-glycans and a single *N*-glycan.^{5a}

On AZT-treated K562 cells, the expression of WGA-recognizing carbohydrate showed an obvious increase of 38%, and PNA-recognizing carbohydrate increased by 21%. As for the carbohydrates corresponding to Con A and DBA, the changes of signals did not give a statistical significance ($p > 0.05$). The results indicated that WGA and PNA-recognizing carbohydrates on cell surfaces were sensitive to AZT treatment, in accordance with previous reports.¹¹ These results demonstrated that the proposed ECL strategy was suitable for highly sensitive and acceptably accurate analysis of dynamic carbohydrate expression on cell surfaces.

In conclusion, a novel ECL cytosensing strategy for sensitive dynamic monitoring of cell surface carbohydrate expression has been successfully designed and demonstrated. Using four

lectins to functionalize the QDs/CS-AuNPs composite film, the resulting interfaces maintain the biological activity of the immobilized proteins and show high ECL intensity. These lectin/QDs/CS-AuNPs modified electrodes act as ECL cytosensors for detection of cell concentration. This ECL strategy for fingerprinting complex cell surface carbohydrate expression has been demonstrated to possess high sensitivity, perfect stability and acceptable reproducibility. This system can be used for analysis of dynamic changes in cell surface carbohydrates in response to drugs, and could be expanded with the addition of more specific glycan-lectin pairs to the repertoire.¹⁵ It could be anticipated that this method would become a powerful tool to decode the complex cell surface carbohydrates and to study the changes occurring in the process of cancer disease progression.

This work was funded by National Natural Science Foundation of China (20821063, 90713015, 20875044), National Basic Research Program of China (No. 2010CB732400), and Natural Science Foundation of Jiangsu (BK2008014).

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Cytosensing and dynamic monitoring of cell surface carbohydrate expression by electrochemiluminescence of quantum dots

En Han, Lin Ding, Hongzhen Lian and Huangxian Ju*

Key Laboratory of Analytical Chemistry for Life Science (Education Ministry of China),

Department of Chemistry, Nanjing University, Nanjing 210093, P.R. China

Experimental

Reagents. Concanavalin A (Con A), wheat germ agglutinin (WGA), *Dolichos bifows* agglutinin (DBA), peanut agglutinin (PNA), bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), chitosan (CS, $\geq 85\%$ deacetylation), hemin, and 3'-azido-3'-deoxythymidine (AZT) were purchased from Sigma-Aldrich Inc. (USA). Fluorescein lectin kit I containing fluorescein isothiocyanate (FITC) labeled lectins (DBA, ConA, PNA, and WGA) was purchased from Vector Laboratories Inc. (USA). Chloroauric acid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$), trisodium citrate and $\text{K}_2\text{S}_2\text{O}_8$ were obtained from Shanghai Reagent Company (Shanghai, China). Thioglycolic acid (TGA) was from Alfa Aesar. All other reagents were of analytical grade. Phosphate buffered saline (PBS, 0.01 M, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.7 mM Na_2HPO_4 and 1.4 mM KH_2PO_4 . All aqueous solutions were prepared using $\geq 18 \text{ M}\Omega$ ultra-pure water (Milli-Q, Millipore).

Cell culture and cell treatment. K562 cell line was kindly provided by Affiliated Zhongda Hospital of Southeast University, Nanjing, China. K562 cells were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin ($100 \mu\text{g mL}^{-1}$), and streptomycin ($100 \mu\text{g mL}^{-1}$) at 37°C in a humidified atmosphere containing 5% CO_2 . The cells in the

exponential growth were collected and separated from the medium by centrifugation at 1000 rpm for 5 min, and then washed thrice with sterile 0.01 M pH 7.4 PBS. The sediment was re-suspended in 0.01 M pH 7.4 PBS to obtain a homogeneous cell suspension. Cell number was determined using a Petroff-Hausser cell counter (USA). The K562 cells were differentiated to erythroid lineages for 4 days by addition of 30 μ M hemin in culture medium. AZT-treated K562 cells were obtained by incubating the cells in culture medium in the presence of 20 μ M AZT for 3 h.

Preparation of TGA-capped CdSe QDs. The water-soluble CdSe QDs were prepared using TGA as stabilizing agent according to a method similar to that reported previously¹ and characterized by UV-vis absorption and photoluminescence (PL) spectroscopy. The Se source was obtained from the reaction between Se powder and NaBH₄ in air-free water. After refluxed at 100 °C for 4 h, the formed CdSe colloid was dialyzed exhaustively against water overnight at room temperature to obtain CdSe QDs solution. Finally, the product was condensed by ultrafiltration at 10000 rpm for 10 min, and the upper phase was decanted and kept at 4 °C.

Preparation of the electrochemiluminescent (ECL) cytosensor. The gold nanoparticles (AuNPs) with a diameter of 13 nm were prepared by reducing chloroauric acid with trisodium citrate according to the previous protocol.² CS solution (0.5% w/w) was prepared by ultrasonically dissolving CS powder in 1% acetic acid, and CS-AuNPs composite solution was obtained by mixing CS and AuNPs solutions at the volume ratio of 2:1. The glassy carbon electrode (GCE) with 5-mm diameter was polished to a mirror using 1.0, 0.3 and 0.05 μ m alumina slurry (Beuhler) followed by rinsing thoroughly with deionized water. After successive sonication in 1:1 nitric acid, acetone and deionized water, the electrode was rinsed with deionized water and allowed to dry at room temperature. CS-AuNPs composite solution (12 μ L) was dropped on the pretreated GCE and dried in the air. The resulting CS-AuNPs/GCE was then immersed in a solution containing 20 μ M CdSe QDs and 5 mM EDC for 5 h at room temperature to obtain QDs/CS-AuNPs/GCE. After rinsing with ultra-pure water, the electrode was dipped into a solution containing 5 mM EDC, 8 mM NHS and a lectin at an optimal concentration for 3 h to yield lectin functionalized QDs on electrode surface. Following a rinse with 0.01 M pH 7.4 PBS, the modified electrode was soaked in 50

mM pH 7.4 Tris-HCl buffer containing 1% BSA and 0.1 M NaCl for 30 min to block the surface active sites for excluding the nonspecific adsorption of cells or other biomacromolecules. 20 μL of 2.0×10^6 cells mL^{-1} K562 cell suspension was dropped on the lectin-immobilized electrode and incubated at 25 $^\circ\text{C}$ for 1 h. After carefully rinsing with 0.01 M pH 7.4 PBS to remove the noncaptured cells, the obtained cytosensor was ready for ECL measurement. For Con A-immobilized electrode, 1 mM Ca^{2+} and 1 mM Mn^{2+} was added to the incubation solution to maintain the binding activity of Con A.

ECL analysis of cell surface carbohydrates. The electrochemical and ECL measurements were carried out on a MPI-A multifunctional analytical system (Xi'an Remex Analytical Instrument Ltd. Co.) at room temperature with a conventional three-electrode system comprised of platinum wire as auxiliary electrode, Ag/AgCl (saturated KCl solution) as reference electrode, and a modified GCE as working electrode. The three-electrode system was immersed in 0.1 M pH 7.4 PBS containing 0.1 M $\text{K}_2\text{S}_2\text{O}_8$ and 0.1 M KCl. The cyclic scan was performed in the potential range from 0 to -1.55 V. The ECL emission window was placed in front of the photomultiplier tube (detection range from 300 to 650 nm) biased at -800 V.

Optimization of lectin modification concentration. Lectins were dissolved in 0.01 M pH 7.4 PBS containing 5 mM EDC and 8 mM NHS at different concentrations (from 0.05 mg mL^{-1} to 2.0 mg mL^{-1}), and then the QDs/CS-AuNPs/GCEs were immersed in the mixed solution. After coupling, BSA blocking, and cell capturing steps, the obtained cell-captured electrodes were subjected to ECL measurements.

Examination on the effect of cell concentration. To demonstrate the cell concentration-dependent signal change, the lectin-immobilized electrodes were incubated with 20 μL of K562 cell suspension at certain concentrations from 5×10^3 to 1×10^7 cells mL^{-1} for 1 h at 25 $^\circ\text{C}$. After careful rinsing with 0.01 M pH 7.4 PBS, the electrodes were subjected to ECL measurements.

Flow cytometric analysis of glycan expression pattern on K562 cell surface. K562 cells were collected by centrifugation at 1000 rpm for 6 min at room temperature. After the cells were washed with cold PBS, they were resuspended in PBS at a concentration of 1×10^7 cells mL^{-1} . 50 μL cell suspension was then added to the mixture of 445 μL PBS and 5 μL 2 mg mL^{-1} fluorescein-labeled lectin. For fluorescein-labeled Con A, 1 mM Ca^{2+} and Mn^{2+} were added to the recognition solution. After incubation

for 30 min, the cells were collected by centrifugation at 1000 rpm for 6 min, washed with PBS, resuspended in 500 μ L PBS, and assayed by flow cytometry. Unlabeled K562 cells were used as the negative control for estimation of autofluorescence, and relative cell-associated fluorescent intensity was obtained by subtraction of autofluorescence. For comparison purpose, the obtained relative cell-associated fluorescent intensity was standardized with the molar ratio of fluorescein/protein.

Apparatus The morphologies of the modified surfaces were studied using atomic force microscopy (AFM, Agilent 5500 model, USA) in tapping mode. Electrochemical impedance spectroscopic (EIS) analysis was performed with an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) in 10 mM pH 7.4 PBS containing 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$ and 0.1 M KCl using the same three-electrode system as used in the ECL detection. UV-vis absorption spectrum was recorded with UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co., Japan). Photoluminescence (PL) spectrum was obtained on a Jasco FP 820 fluorometer (Jasco Co., Japan). Flow cytometric analysis was performed on FACSCalibur flow cytometer (Becton Dickinson, USA).

Characterization of TGA-capped CdSe QDs.

The formation of TGA-capped CdSe QDs was characterized by PL and UV-vis spectra of 20-times diluted as-synthesized CdSe QDs solution. The first UV-vis absorption peak occurred at 427 nm ([curve A, Fig. S1](#)), from which the size of the resulting CdSe QDs and the concentration of QD solution could be estimated to be 1.8 nm and 244 μ mol L⁻¹ with the adsorption peak and Peng's empirical equations.³ The PL spectrum (excited at 431 nm) of CdSe QDs solution showed a relatively narrow emission with a maximum intensity at 546 nm ([curve B, Fig. S1](#)).

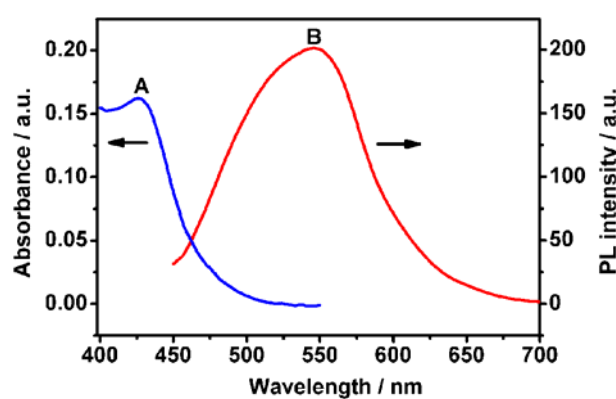


Fig. S1 UV-vis (A) and PL (B) spectra of QDs dissolved in pH 7.4 PBS. Excitation wavelength: 431 nm.

Table S1 Binding specificity of carbohydrate and lectin⁴ and optimal concentrations for immobilization of lectins.

Lectins	Binding specificity ^a	<i>c</i> (mg mL ⁻¹)
Con A	terminal α -Man, Man α 3(Man α 6)Man	1.5
DBA	GalNAc α -Ser/Thr (Tn), GalNAc α 1-3GalNAc	0.5
PNA	Gal β 1-3GalNAc α -Ser/Thr, Gal β 1-3GalNAc β 1-4Gal β	1
WGA	(GlcNAc) _n , multivalent Sia	1.5

^a Man, mannose; GalNAc, *N*-acetylgalactosamine; Gal, galactose; Ser, serine; Thr, threonine; GlcNAc, *N*-acetylglucosamine; Sia, sialic acid.

AFM characterization of electrode at different modification stages

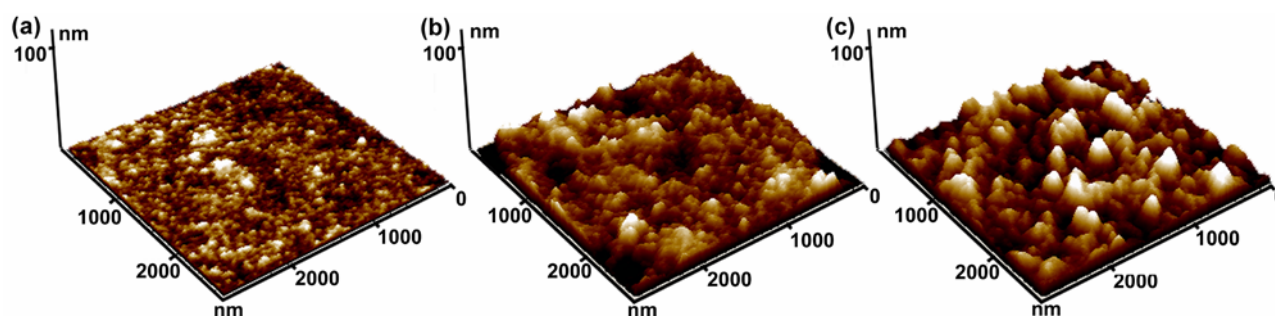


Fig. S2 Topographic images of CS-AuNPs (A), QDs/CS-AuNPs (B) and WGA/QDs/CS-AuNPs (C) films.

EIS characterization of electrode at different modification stages

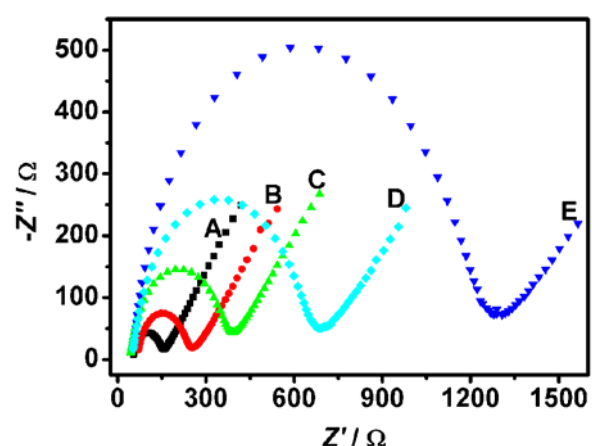


Fig. S3 EIS of bare GCE (A), CS-AuNPs/GCE (B), QDs/CS-AuNPs/GCE (C), WGA/QDs/CS-AuNPs/GCE (D) and cells/WGA/QDs/CS-AuNPs/GCE (E) in 10 mM pH 7.4 PBS containing 0.1 M KCl and 5 mM $K_4Fe(CN)_6/5\text{ mM}K_3Fe(CN)_6$. The frequency range is between 0.05 Hz and 10 kHz with signal amplitude of 5 mV.

Table S2 Intra-assay and inter-assay variation coefficients (CVs) of the ECL cytosensor

Lectins	Intraassay CVs (%)	Interassay CVs (%)
Con A	4.1	7.2
DBA	2.4	5.6
PNA	3.9	6.6
WGA	4.7	8.2

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