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

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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, ≥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 ($HAuCl_4 \cdot 4H_2O$), trisodium citrate and $K_2S_2O_8$ 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 ≥ 18 MΩ 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 μg mL^{-1}), and streptomycin (100 μg 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 μM hemin in culture medium. AZT-treated K562 cells were obtained by incubating the cells in culture medium in the presence of 20 μM 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 previously[1] and characterized by UV-vis absorption and photoluminescence (PL) spectroscopy. The Se source was obtained from the reaction between Se powder and $NaBH_4$ 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 μm 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 μL) was dropped on the pretreated GCE and dried in the air. The resulting CS-AuNPs/GCE was then immersed in a solution containing 20 μM 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 °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 $K_2S_2O_8$ 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 °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 μL 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 FACSCalibar 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 μmol 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).

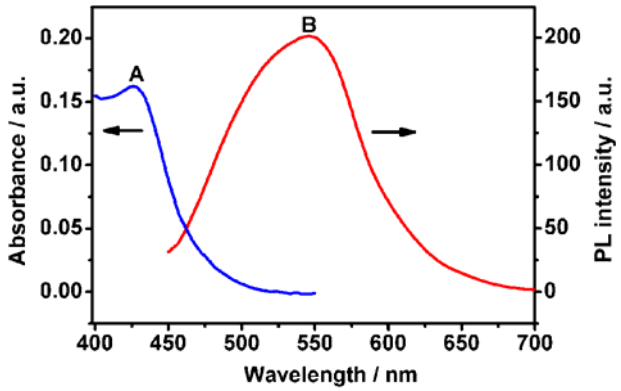

Fig. S1 UV-vis (A) and PL (B) spectra of QDs dissolved in pH 7.4 PBS. Excitation wavelength: 431 nm.

Table S1 Binding specificity of carbohydrate and lectin[4] and optimal concentrations for immobilization of lectins.

Lectins	Binding specificity[a]	*c* (mg mL^{-1})
Con A	terminal α-Man, Manα3(Manα6)Man	1.5
DBA	GalNAcα-Ser/Thr (Tn), GalNAcα1-3GalNAc	0.5
PNA	Galβ1-3GalNAcα-Ser/Thr, Galβ1-3GalNAcβ1-4Galβ	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

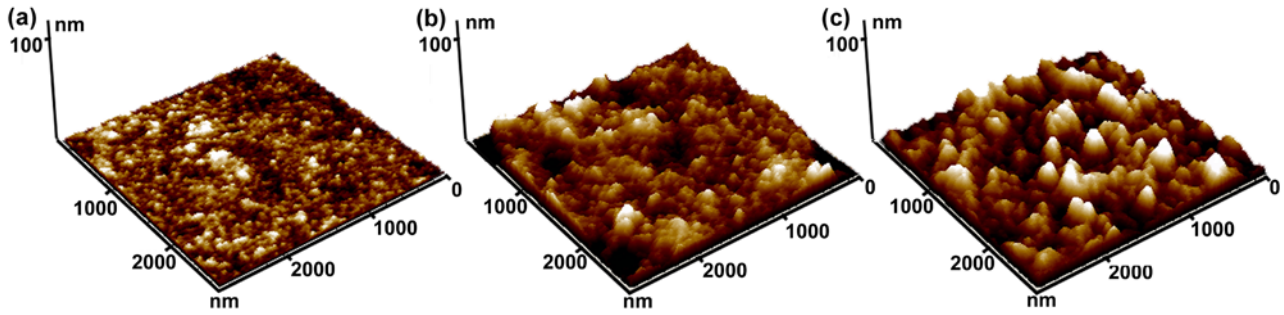

Fig. S2 Topographic images of CS-AuNPs (A), QDs/CS-AuNPs (B) and WGA/QDs/CS-AuNPs (C) films.

EIS characterization of electrode at different modification stages

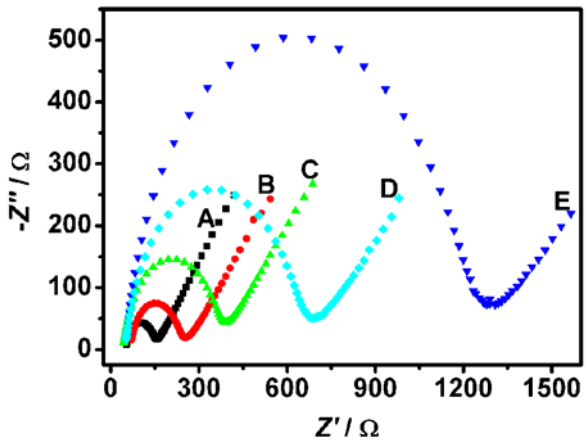

Fig. S3 EIS of bare GCE (A), CS-AuNPs/GCE (B), QDs/CS-AuNPs/GCE (C), WGA/QDs/CS-AuNPs/GCE (D) and cells/WGA/QDs/CS-AuNPs/GCE (E) in 10 mM pH 7.4 PBS containing 0.1 M KCl and 5 mM $K_4Fe(CN)_6$/5 mM$K_3Fe(CN)_6$. The frequency range is between 0.05 Hz and 10 kHz with signal amplitude of 5 mV.

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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