A simple fluorescent strategy for in situ evaluation of cell surface carbohydrate with quantum dot-lectin nanoprobe

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Experimental

Reagents. Concanavalin A (Con A), mercaptopropionic acid (MPA), bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), swainsonine (SW) were purchased from Sigma-Aldrich Inc. (USA). Fluorescein lectin kit I containing fluorescein isothiocyanate (FITC) labeled lectin (Con A) was purchased from Vector Laboratories Inc. (USA). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) was purchased from Alfa Aesar China Ltd. Tellurium powder and sodium borohydride with analytical grade were from Sinopharm Chemical Reagent Co., Ltd (China). Coomassie brilliant blue G-250 (CBBG) was purchased from Jiangsu Qiangsheng Chemical Co., Ltd (China). 0.01 M pH 7.4 phosphate buffered saline (PBS) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4 and 1.41 mM KH_2PO_4. All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (≥ 18 MΩ, Milli-Q, Millipore).

Cell culture and cell treatment. K562 cell line was kindly provided by Affiliates Zhongda Hospital, 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 1,000 rpm for 5 min, and then washed thrice with sterile 0.01 M pH 7.4 PBS. The sediment was re-suspended in the PBS to obtain a homogeneous cell suspension. Cell number was determined using a Petroff-Hausser cell

counter (USA). The culture conditions of BGC-823 cells were the same as those of K562 cells. SW-treated K562 cells were obtained by incubating the cells in culture medium in the presence of 2 μg mL^{-1} SW for 56 h.

Preparation of MPA-modified CdTe QDs. Briefly, 26 μL of MPA was added to 50 mL of 2.0 mM $CdCl_2$ solution. After adjusting the pH to 9.0 with 1 M NaOH, the resulting clear solution was bubbled with highly pure N_2 for 30 min. A 0.80 mL NaHTe solution of 0.0625 M, obtained by mixing oxygen-free $NaBH_4$ solution with Te powder, was then slowly injected into the vigorously stirred and oxygen-free MPA-$CdCl_2$ mixed solution. The molar ratio of Cd^{2+}/MPA/HTe^- were 1:3:0.5. The obtained solution was refluxed for 10 h to produce the QDs of 2.8 nm in diameter. The resulting QD solution was rather stable in at least 3 months when was kept in a refrigerator at 4 °C, free of light. The QD solution was subjected to ultrafiltration to remove excessive MPA using Vivaspin concentrator (Sartorius, 10,000 MW) at 15,000 g for 10 min at 4 °C. The upper phase was washed three times with water, and then diluted to 700 μL with pH 7.4 PBS, which was measured with UV-vis method to be 7.6 μM.

Preparation of QD-Con A nanoprobe. 700 μL MPA-derivatized CdTe QD solution (7.6 μM) was mixed with the mixture of EDC (1 mg in 50 μL PBS) and Con A (250 μL, 1 mg mL^{-1} in PBS) for conjugation of QDs and Con A. After incubation for 3 h at 25 °C under shaking and free of light, the resulting sample was ultrafiltrated using Vivaspin concentrator (Sartorius, 100,000 MW) at 3,000 g for 12 min at 4 °C to remove the non-conjugated QDs and by-prodect. The obtained nanoprobe was washed with 50 mM pH 7.4 Tris-HCl buffer and then 10 mM pH 7.4 PBS for three times by ultrafiltration. The formed nanoprobe was diluted to 700 μL and kept at 4 °C.

Concentration detection of QD-Con A nanoprobe. The concentration of QD-Con A was analyzed according to Bradford method. CBBG (100 mg) was dissolved in 50 mL 95% ethanol, which was then mixed with 100 mL 85% (w/v) phosphoric acid and 850 mL water to obtain CBBG solution. 1 mL protein solutions containing 20, 40, 60, 80, 100 μg Con A were added to 5 mL CBBG solution in 12×100 mm test tubes, respectively. After 2-min vortexing, the absorbance of the mixture at 595 nm was

measured to obtain a standard curve for the determination of protein concentration on the synthesized nanoprobe.

Fluorescent detection of cell surface carbohydrate expression. The fluorescent intensity of solutions containing 0.54, 0.72, 0.9, 1.08, 1.26, 1.44, 1.62, 1.8 μM QD-Con A nanoprobe, 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} were measured in 0.5 mL cuvettes, respectively, to obtain a standard curve for the determination of nanoprobe concentration in homogeneous supernatant.

For cell surface mannosyl group detection, 0.5 mL QD-Con A nanoprobe at an optimal concentration was incubated with 500 μL 1×10^4 cells mL^{-1} K562 cells in the presence of 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} under gentle shaking at 25 °C for 1 h. Afterwards, the supernatant was collected by centrifugation at 1,000 rpm for 10 min to carry out the fluorescent measurement. The change of fluorescent intensity upon cell incubation was due to the binding of QD-Con A nanoprobe to cell surface. The average amount of mannose moieties on cell surface could thus be estimated.

Flow cytometric analysis of glycan expression pattern on K562 cell surface. K562 cells were collected by centrifugation at 1,000 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 1,000 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.

Apparatus. The UV-vis absorption spectra were obtained with UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Kyoto, Japan). Infrared spectra were recorded on a Nicolet 400 Fourier transform infrared (FT-IR) spectrometer (Madison, WI) using solid samples, which were prepared by drying under infrared lamp. The fluorescent intensity was measured by RF-5301PC spectrofluorophotometer (Shimadzu, Japan). Fluorescence images of cells were taken by TE200-U inverted fluorescence microscopy (Nikon, Japan).

Flow cytometric analysis was performed on FACSCalibar flow cytometer (Becton Dickinson, USA). All the experiments were performed at 25 °C.

Characterization of the QD-Con A nanoprobe

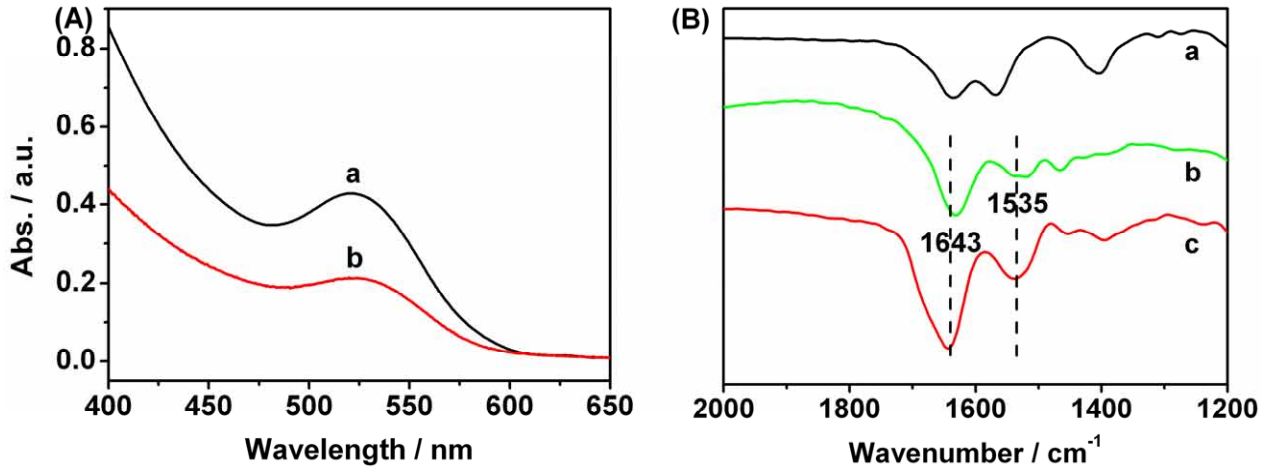

Fig. S1 (A) UV-vis absorption spectra of 4.7 μM QDs (a) and 6.6 μM QD-Con A nanoprobe (b); (B) IR spectra of pure QDs (a), pure Con A (b) and QD-Con A nanoprobe (c).

UV-vis analysis of QD-Con A nanoprobe concentration

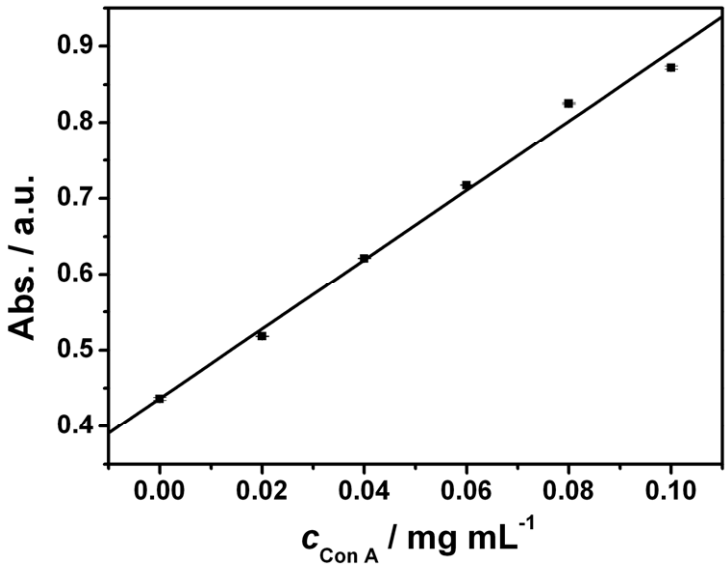

Fig. S2 Linear calibration plot of absorbance of CBBG mixed Con A solution *vs*. concentration of Con A (R = 0.996, n = 6).

Optimization of recognition time

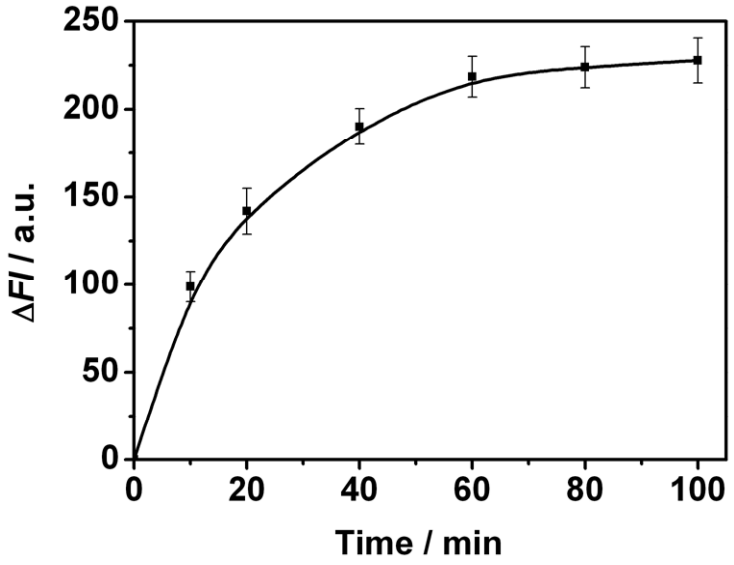

Fig. S3 Plot of Δ*FI* vs. recognition time using 1.4 μM QD-Con A nanoprobe for incubation with cells.