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Supporting Information

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A Facile Electrochemical Cytosensor Array for Dynamic Analysis of Carcinoma Cell Surface Glycans

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Experimental Details

Materials and Reagents. Horseradish peroxidase labeled concanavalin A (HRP-ConA), horseradish peroxidase labeled wheat germ agglutinin (HRP-WGA), horseradish peroxidase labeled dolichos bifows agglutinin (HRP-DBA), horseradish peroxidase labeled peanut agglutinin (HRP-PNA), bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 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). Arginine-glycine-aspartic acid-serine tetrapeptide (RGDS) was obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). Single-walled carbon nanotubes (SWNTs) were purchased from Shenzhen Nanotech Port Ltd. Co. (China). Phosphate buffer saline (PBS, pH 7.4) 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).

To obtain carboxylic group-functionalized SWNTs, the received SWNTs were firstly dispersed in 30% HNO_3 and refluxed for 24 h at 140 °C. After the resulting suspension was centrifuged, and the

sediment was washed with deionized water until the pH reached 7.0, the functionalized SWNTs were dispersed in deionized water to a concentration of 1 mg mL^{-1}.[S1]

Cell Culture and Treatment. K562 cell line was kindly provided by the Affiliated 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 phase were collected and separated from the medium by centrifugation at 1000 rpm for 5 min, and then washed thrice with a sterile pH 7.4 PBS. The sediment was re-suspended in the PBS containing 1 mM Ca^{2+} and 1 mM Mg^{2+} to obtain a homogeneous cell suspension. Cell number was determined using a Petroff-Hausser cell counter (USA). AZT treated K562 cells were obtained by incubating the cells in culture medium containing 20 μM AZT for 3 h. The K562 cells were induced to differentiate for 1-7 days by addition of 1 mM sodium butyrate (NaBu) in culture medium, respectively.

Preparation of Screen-printed Carbon Electrodes. The screen-printed carbon electrodes (SPCEs) were fabricated according to the steps reported previously.[S2] Firstly the silver ink was screen-printed on a nylon sheet as conductive bands. Then the graphite ink was imprinted to cover the areas that server as four working electrodes and one auxiliary electrode, and the silver chloride ink was imprinted on the area of reference electrode. Finally, the conductive bands were insulated by overlaying an insulating dielectric material except the electric connectors and the electrode areas (2 mm diameter, 0.5 mm edge-to-edge separation). All working electrodes shared the same reference and auxiliary electrode.

Preparation of RGDS-SWNTs/SPCE. 1 μL of 1 mg mL^{-1} carboxylic group-functionalized SWNTs solution was dropped on each graphite working electrode of SPCEs, respectively. After dried in a desiccator, 1 μL solution containing 2 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 5 mM N-hydroxysuccinimide (NHS) was dropped on each obtained SWNTs/SPCE and incubated for 1 h. After the activated SWNTs/SPCEs were thoroughly rinsed with deionized water, 1 μL of 1 mg mL^{-1}

RGDS was immediately coated on each SWNTs/SPCE and incubated for 2 h to obtain RGDS-SWNTs/SPCEs for cell capture.

Apparatus and Characterization. All electrochemical measurements were performed on a CHI 660 electrochemical analyzer (Co. CHI, TX). An electronic switch was used for simultaneous 4-analyte detection. The differential pulse voltammetric measurements were performed from -0.4 to -1.0 V with pulse amplitude of 50 mV and width of 50 ms in degassed 0.1 M pH 7.0 PBS containing 8.0 mM H_2O_2 and 10 mM o-PD. The morphologies of RGDS-SWNTs/SPCE and bare SPCE were observed under a Hitachi S-4800 scanning electron microscopy (SEM, Japan). Flow cytometric analysis was performed on FACSCalibur flow cytometer (Becton Dickinson, USA) .

Optimization of Cell Concentration for Capture Step. The RGDS-SWNTs/SPCE was incubated with 1 μL K562 cell suspension at certain concentrations from 2.5×10^5 to 1×10^7 cells mL^{-1} at 37 °C for 1 h. After carefully rinsing and blocking, the resulting cytosensors were incubated with 1 μL of 60 μg mL^{-1} HRP-WGA at room temperature for 1 h and subjected to DPV measurements.

Optimization of HRP-lectins Concentration. HRP-Lectins (HRP-ConA, HRP-WGA, HRP-DBA, HRP-PNA) were dissolved in 0.01 M pH 7.4 PBS at different concentrations (from 10 to 70 μg mL^{-1}), and then 1 μL of the solutions were dropped on K562 each cytosensor, respectively. After incubation at room temperature for 1 h and carefully rinsing, the obtained cytosensors were subjected to DPV measurements.

Benzidine Staining Experiments. 50 μL cell suspension at 1×10^6 cells mL^{-1} was mixed with 10 μL benzidine reagent containing 0.6% H_2O_2, 0.5 M acetic acid and 0.2% benzidine dihydrochloride and incubated for 10 min. The percentage of benzidine staining-positive cells was determined by light microscopic examination. Each experiment was performed in triplicate, and the results were averaged.[S3]

Flow Cytometric Analysis. K562 cells in the exponential growth phase were collected and separated from the medium by centrifugation at 1000 rpm at room temperature for 6 min. Subsequently, the cells

were washed with sterile cold pH 7.4 PBS, re-suspended in PBS, and the cell concentration was determined. Then, 50 μL of 1×10^7 cells mL^{-1} cell suspension and 445 μL of pH 7.4 PBS were mixed with 5 μL of 1 mg mL^{-1} fluorescein isothiocyanate (FITC) labeled lectins (DBA, ConA, PNA, and WGA), and incubated at room temperature for 30 min, respectively. The cells were collected by centrifugation at 1000 rpm for 6 min, washed twice with 200 μL cold PBS, and were re-suspended in 500 μL of PBS and assayed by flow cytometry. Unlabeled cells were used for estimation of autofluorescence. [S4]

Table S1. Glycan-binding specificities of the lectins used in this paper.[S5]

Lectins	Origin	Binding Specificity[a]
ConA	*Canavalia ensiformis*	Terminal α Man, Man α3[Man α6] Man
DBA	*Dolichos biflorus*	GalNAc α1,3 GalNAc/Gal
PNA	*Arachis hypogaea*	Gal β(1,3)GalNAc, Terminal βGal
WGA	*Triticum unlgari*	(Neu5Ac) (Gal β4GlcNAc)$_{1\text{-}3,4}$ (GlcNAc β4GlcNAc)$_{1\text{-}3,4}$

[a] Man, mannose; GalNAc, *N*-acetylgalactosamine; Gal, galactose; GlcNAc, *N*-acetylglucosamine; Neu5Ac, *N*-acetylneuraminic acid.

SEM Characterization of RGDS-SWNTs/SPCE.

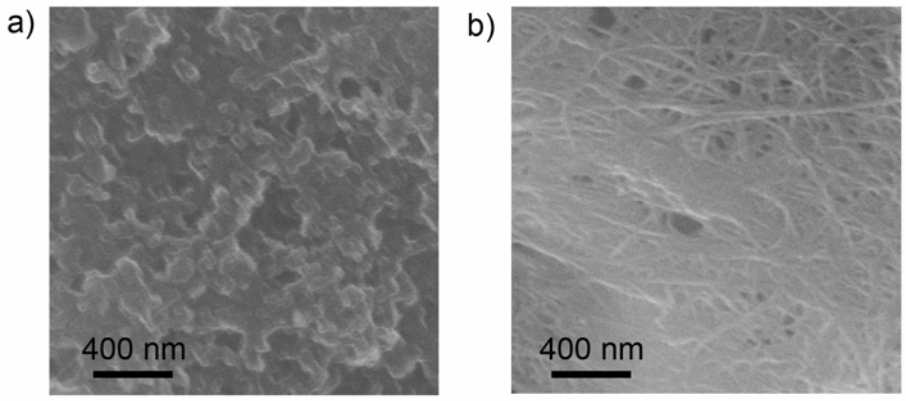

Figure S1. SEM images of a) bare SPCE and b) RGDS-SWNTs/SPCE.

Cyclic Voltammetric Characterization of Cytosensor.

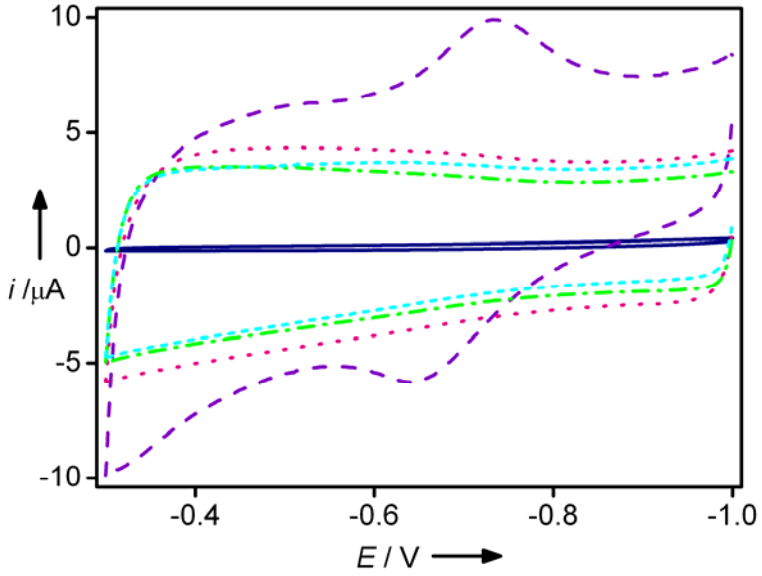

Figure S2. Cyclic voltammograms of SPCE (solid, navy), RGDS-SWNTs/SPCE (short dot, cyan), K562/RGDS-SWNTs/SPCE (dash dot, green), and HRP-WGA/K562/RGDS-SWNTs/SPCE (dot, magenta) in 0.1 M pH 7.0 PBS and HRP-WGA/K562/RGDS-SWNTs/SPCE in 0.1 M pH 7.0 PBS containing 8.0 mM H_2O_2 and 10 mM *o*-PD (dash, purple). Scan rate: 100 mV s^{-1}.

Flow Cytometric Analysis of Glycan Expressoin on K562 Cell Surface

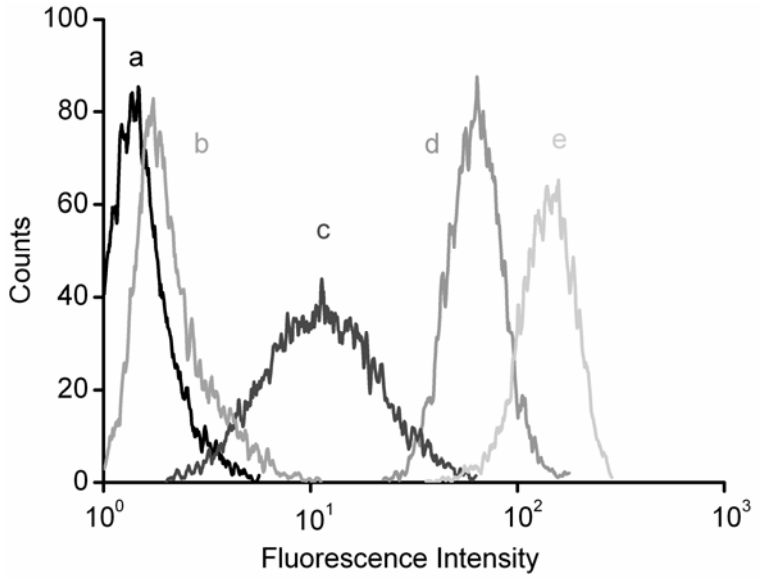

Figure S3. Fluorescence profiles of FITC-lectins treated K562 cells. (a) autofluorescence, (b) FITC-DBA, (c) FITC-PNA, (d) FITC-ConA and (e) FITC-WGA analyzed by flow cytometry.

Reproducibility of the array

Table S2. Intra-assay and inter-assay CVs of the array

Lectins	Intra-assay CVs (%)	Inter-assay CVs (%)
ConA	4.76	7.56
WGA	4.94	7.61
PNA	2.50	5.76
DBA	3.64	5.84

Comparison of Glycan Expression Patterns on K562 and AZT Treated K562 Cells.

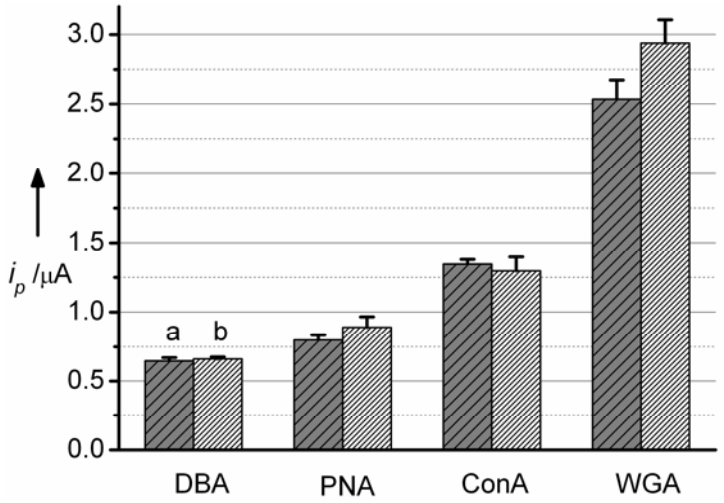

Figure S4. DPV responses of four HRP-lectins bound on K562 (a) and AZT treated K562 cells (b).

Supporting references:

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[S3] N. Belhacène, L. Maulon, S. Guérin, J. E. Ricci, B. Mari, Y. Colin, J. P. Cartron, P. Auberger, *FASEB J.* **1998**, *12*, 531.

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[S5] K. A. Wearne, H. C. Winter, K. O'Shea, I. J. Goldstein, *Glycobiology* **2006**, *16*, 981.