Supporting Information

Ultrasensitive Photoelectrochemical Biosensing of Cell Surface N-glycan Expression Based on the Enhancement of Nanogold-assembled Mesoporous Silica Amplified by Graphene Quantum Dots and Hybridization Chain Reaction

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Reagents and Material

Ultrapure water obtained from a Millipore water purification system (resistivity ≥ 18.2 MΩ·cm, MilliQ, Millipore) was used in all assays and solutions. Carbon ink (ED423 ss) and Ag/AgCl ink (CNC-01) were obtained from Acheson. Whatman chromatography paper #1 was purchased from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size (A4 size). Tetraethoxysilane (TEOS), cetyl trimethyl ammonium bromide (CTAB), Poly (vinylpyrrolidone) (PVP, K-30), tetrachloroauric acid ($HAuCl_4 \cdot 4H_2O$), horseradish peroxidase (HRP), Luminol, concanavalin A (Con A)，H_2O_2 were purchased from Sigma-Aldrich Chemical Co. Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), ammonium hydroxide, and cadmium chloride were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Cysteamine (CA) and Glutaraldehyde (GA, 25% aqueous solution) were purchased from Alfa Aesar ChinaLtd. The synthetic oligonucleotides used in this study were purchased from Sangon Biotech Co., Ltd. (Jinan, China) and its sequences are given below:

I:

5’-**CTAGAGCACAATCACAGGAGCCAG**TTTTTT-COOH-3’

H1:

5’-*GCAGTTGATCCTTTGGATACCCTGG*TTTT**CTGGCTCCTGTGATTGTGCT**

CTAGTTTACATCGCTAGAGCACAATCACAGG–biotin-3’

H2:

5’-*GCAGTTGATCCTTTGGATACCCTGG*TTTT**CTAGAGCACAATCACAGGAG**

CCAGTTTCCTGTGATTGTGCTCTAGCGATGT-biotin-3’

The sequence expressed as italics was the aptamer of the MCF-7 cells. The sequence expressed as bold (a single underline) is complementary to the sequence expressed as bold (a single underline).

Apparatus and Characterization

The photocurrent was measured by the current-time curve experimental technique on a CHI660D electrochemistry workstation (Shanghai CH Instruments Co., China) with a three-electrode system: a modified paper-based electrode as the working electrode, a printing carbon as the counter electrode, and an Ag/AgCl electrode as the reference electrode. Electrochemical impedance spectroscopy (EIS) were performed using an IM6x electrochemical work station (Zahner Co., Germany). PEC measurements were performed with a homemade PEC system. Transmission electron microscope (TEM) images were obtained from a JEOL JEM-1400 microscope (JEOL, Japan). Scanning electronmicroscopy (SEM) images were performed with a QUANTA FEG 250 thermal field emission scanning electron microscopy (FEICo., USA). Infrared spectroscopy (IR) were obtained on a Fourier transform infrared (FT-IR) Spectrum RX (PerkinElmer Spectoment). Ultraviolet-visible (UV-vis) absorption spectra were recorded on a UV-2550 spectrophotometer (Shimadzu,

Japan).

Cell Culture and Cell Treatment

The MCF-7 (human breast carcinoma) cell line was kindly provided by Shandong Tumor Hospital. The MCF-7 cells were cultured and maintained in dulbecco's modified eagle medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The culture medium was changed every other day and the cells were passaged when they reached 80-90% confluency. MCF-7 cells were separated from the medium by centrifugation at 1000 rpm for 5 min when the cells were grown to midlog phase, and washed with sterile Dulbecco's phosphate-buffered saline (DPBS, pH 7.4) twice. The supernatant was aspirated, and the cells were re-suspended in the binding buffer (4.5 g L^{-1} glucose, 5 mM $MgCl_2$ and 1mg mL^{-1} BSA, all dissolved in Dulbecco's PBS with $CaCl_2$ and $MgCl_2$) to obtain a homogeneous cell suspension which was used to ensure the effective binding affinity between the cells and the aptamer.

The MCF-7 cells were seeded to culture plate at the density of 1×10^5 cells mL^{-1} and then incubated in the culture medium respectively containing 1.2 μM (approximate 1 μg mL^{-1}) tunicamycin (TM) or 500 μM benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (BG) for 48 h for evaluating the inhibition effect of the cell surface carbohydrate expression under different external stimulation. The N-glycans was cleaved under the effect of 2000 NEB units of peptide-N-glycosidase F (PNGase F) in the suspension containing 2% BSA via mildly

shaken for 24 h. In addition, the MCF-7 cells primarily inhibited by 1.2 μM TM or 500 μM BG for 48 h were also chosen for enzymatic digestion following the same procure above.

Synthesis of Porous ZnO spheres

Briefly, 2.5 g of soluble starch was dissolved initially in 75 mL of boiling ultrapure water. The obtained mixture was stirred at 85 ºC for 5 min after the addition of 0.005 mol of $Zn(NO_3)_2 \cdot 6H_2O$. Then the pH of the solution was adjusted to 8-9 by adding ammonium hydroxide dropwise and kept on stirring for an additional 30 min at 85 ºC. Following that, the resulting precipitate was centrifuged, washed with ultrapure water, and dried at 50 ºC. Finally, the as-obtained powders were calcined in air atmosphere at 500 ºC for 2 h to achieve porous ZnO spheres.

The porous ZnO treated by 4-aminothiophenol (PATP) was prepared according to the following procedure.[1] ZnO were dissolved in 0.1mM PATP ethanol solution and the mixture was stirred for 4 h. Then the precipitate was centrifuged and rinsed with ethanol three times.

Synthesis of Graphene Quantum Dots (GQDs) and CdTe-NH_2 QDs

In Brief, 0.25 g of XC-72 carbon black was sonicated for 1 h in 50mL of 6 mol $\cdot L^{-1}$ HNO_3, then the suspension was refluxed for 24 hat 130 °C. After cooling to room temperature, the suspension was centrifuged for 30min at 10000 rpm to obtain a yellow supernatant. The GQDs were obtained as a reddish-brownsolid after the

supernatant was heated at 100 ℃ removing water and nitric acid. Subsequently, the solid was dissolved in water and dialyzed in a dialysis bag for 3 days. Afterdialysis, the solution was centrifuged for 30 min. Finally, the GQDs were kept at 4 ℃prior to use.

To synthesize CdTe-NH_2, 3.6 mmol of CA was mixed with $CdCl_2$ solution (12 mM, 100 mL) under stirring. Then the pH of the solution was adjusted to 5.7 by dropwise addition of 1.0 M NaOH. Subsequently, 0.1 g of $NaBH_4$ and 0.06 mmol of Na_2TeO_3 were successively added in the solution deaerated with highly pure N_2 for 30 min. The typical molar ratio of Cd^{2+}: Te^{2-} : CA was 1: 0.05 :3. Under nitrogen atmosphere, the mixture solution was refluxed at 100 ℃ for 1 h, and eventually the CdTe-NH_2 solution was obtained.[2]

Preparation of mesoporous silica nanoparticles (MSNs) and nanogold assembled MSNs(GMSNs)

MSNs, as the carriers of nanogold, were synthesized according to previous report with some modifications. In brief, 0.20 g of CTAB was dissolved in 100 mL of distilled water. With continue stirring, 700 μL of sodium hydroxide (2.0 M) was added into CTAB solution at 80 ℃ for 20 min. Then, 1.00 mL of TEOS was injected into the above solution, and vigorously stirred for additional 2 h. Following that, the obtained product was centrifuged, washed with deionized water and methanol, and dried in air. Subsequently, 0.164 g of the precipitates was refluxed for 10 h in a mixture containing 1.5 mL of HCl (37%) and 75 mL of methanol to remove the surfactant template. The

obtained MSNs were centrifuged, washed with distilled water and methanol, and dried for 4 h at 60 ºC.

The utilized GMSNs was fabricated as follow: 10 mg of MSNs was initially dispersed in PVP aqueous solution (0.5 wt %, 10 mL), and then kept the mixture on stirring for 2 h at room temperature to make the positively charged PVP adsorb on the negatively charged MSNs adequately. Afterward, the resulting mixture was centrifuged for10 min at 12000 rpm, then the obtained precipitate was dispersed in $HAuCl_4$ solution (1.0 wt %, 2 mL). The mixture was shaken slightly for 6 h at room temperature to make the $[AuCl_4]^-$ ions assemble on the surface of the PVP functionalized MSNs. Subsequently, the Au (III) was reduced to zero valent gold nanoparticles by using 0.2 M glucose solution at 95 ºC.[3] The resultant GMSNs were collected by centrifugation, and dispersed into 5 mL of pH 7.4 Tris buffer solution to use.

Preparation of GQDs@Con-A nanoprobes

The 800 μL of as-prepared GQDs suspension and Con-A aqueous solution (100 μL, 2 mM) were added to a H_2O-diluted solution (10.0 mL) and slightly stirred mechanically overnight at 4 °C to obtain GQDs@Con-A nanoprobes. The obtained precipitate were centrifuged, washed with distilled water and dispersed with 1.0 mL incubation buffer containing 0.1 mM Mn^{2+} to remain binding activity of Con-A. Then the GQDs@Con-A bioconjugates were storedat 4 °C for further use.

Fabrication of the Au Nanoparticles (Au NPs) Modified Paper Working Electrode (Au-PWE)

The 3D paper-based PEC origami device was fabricated according to the previously reported method.[4]The Au-PWE with excellent conductivity and enlarged surface area was obtained through growth of Au nanorod layer on the surfaces of cellulose fibers according to our previous works with slight modifications.[5] Firstly, the Au NPs seeds was obtained by using $NaBH_4$ as reductant and stabilized with srodium citrate according to the literature.[6] Then, 15 μL of the achieved Au NPs seeds solution was injected into PWE. After 90 min, the process of rinsing with water was repeated five times to remove loosely bound Au NPs seeds. Subsequently, freshly prepared aqueous growth-solution (15 μL) of PBS (10.0 mM, pH 7.0) containing $HAuCl_4$ (1.2×10^{-3} M), cetyltrimethyl ammonium chloride (3×10^{-3} M), and H_2O_2 (8.0×10^{-3} M) for seeds growth was dropped to the Au NPs seeded PWE respectively with an incubation at room temperature for 15 min. Subsequently, the resulting Au-PWE were washed with water thoroughly. Thus the layer of interconnected Au nanorod on cellulose fibers with excellent conductivity were obtained, which were dried at room temperature for 30 min.

Procedure of formation for HRP labled double helices DNA with multiple branched arms (HRP-mdhDNA)

The synthesis of HRP-mdhDNA conjugateswas carried out similar to the reported method created by Pierce's group.[7] In details, initiator strand (I),stock

solutions of H1 and H2 were dissolved in TM buffer (10 mM Tris-HCl and 50 mM $MgCl_2$, pH 8.0), yielding a final concentration of 5 μM, 20 μM and 20 μM respectively. Subsequently,they were heated to 95 ºC for 5 min and cooled to 4 ºC within 30 s by use of a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA). Then, 100 μL of I solution, 500 μL of H1 and 500 μL of H2 were mixed with approximate final concentrations of 0.5, 10 and 10 μM for I, H1 and H2, respectively. The prepared hybridization mixture was reacted overnight at 37 ºC and formed double strands DNA with multiple branched arms that could recognise the target cells specifically. The HRP-mdhDNA was formed by mixing the resultant mdhDNA with avidin-HRP (10 μg/mL) for 15 min, then the products were centrifuged, washed with distilled water and dispersed in 2 mL TM buffer solution and stored at 4 ºC prior to use.

Fabrication of the PEC Detection Area

Firstly, the porous ZnO solution treated by PATP was dropped onto the Au-PWE surface and formed Au-PWE/ZnO electrode. After incubated for 2 h at room temperature, the electrode was carefully washed with doubly distilled water and dried at room temperature. In this process, the formation of Zn-S bond made ZnO posses remaining amino groups of PATP molecules, which could bind to the Au-PWE and GQDs [8].

Then the electrode anchored ZnO was activated with 2.5% GA for 30 min and washed with water. Subsequently, 25 μL of as-prepared CdTe QDs was spread onto

the resulting electrode surface in a moisture atmosphere to avoid evaporation of solvent. After incubation for 35 min, the electrode was rinsed with the washing buffer to remove excess CdTe QDs.

After the obtained electrode was activated by 2.5% GA for 30 min, the GMSNs treated by ethylenediamine was immobilized onto the Au-PWE/ZnO electrode modified by CdTe QDs and formed Au-PWE/ZnO/CdTe/GMSNs, and the procedure was described below. In detail, 2.0 mL GMSNs and 4.0 mL ethylenediamine were first dispersed in 10 mL water by sonication for 1 h. After stirring for 5 h, the suspension was centrifuged and washed with water repeatedly for three times. Then the as-prepared amino-functionalized GMSNs was diluted to 2 mL with 0.1 M Tris-HCl buffer .The 25 μL amino-functionalized GMSNs was dropped on the prepared electrode with a incubation at 4 ℃ for 35 min, then the electrode was rinsed with the washing buffer to remove the unbounded GMSNs.

Then the 50 μL as-prepared HRP-mdhDNA solusion was dropped on the electrode activated using the classic EDC coupling reaction and reacted at 37℃ for 1 h. Then the electrode was rinsed with the washing buffer to thoroughly removing the excess HRP-mdhDNA. Subsequently, a drop of 10 μL 2% BSA solution was added to the as-obtained electrode allowed to react at room temperature for 6 min to ensure the blocking of nonspecific binding sites.

In order to capture the cells, 20 μL of homogeneous MCF-7 cells suspension at a certain concentration was dropped into the as-obtained electrode and incubated at

37 ℃ for 40 min. Then the cells were captured on the surface of the electrode though the specific binding between the aptamers and cells. Subsequently, the cell captured electrode was incubated with 80 μL of as-prepared GQDs@Con A for 1 h at 37℃ through the specific recognition the mannose on of captured cell surfaces by Con-A and then was rinsed with PBS solution. After that, the electrode was rinsed with PBS solution containing luminol and H_2O_2 for PEC measurements.

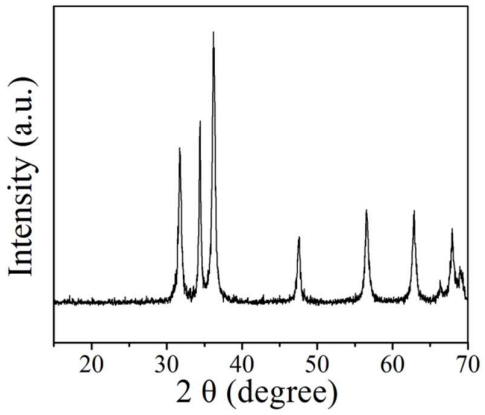

Figure S1. XRD pattern of the ZnO.

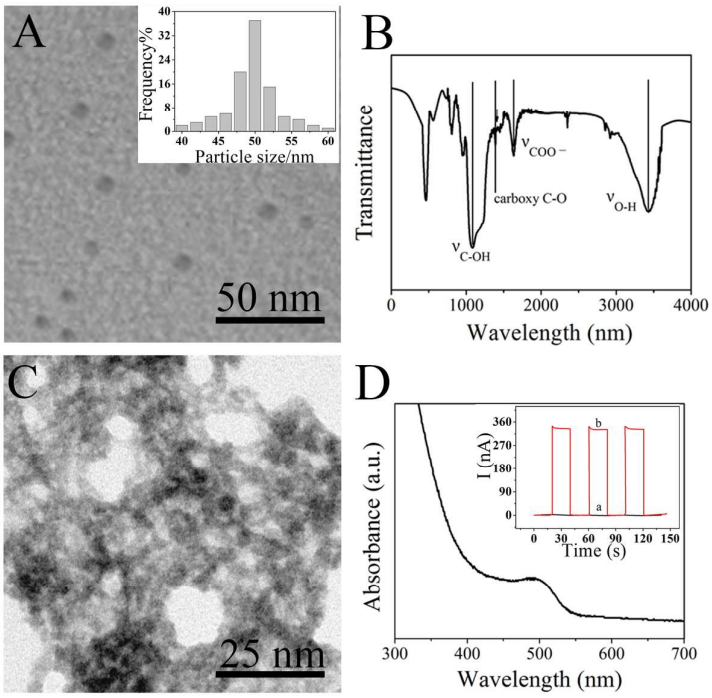

Figure S2. TEM image (A) and FT-IR spectra (B) of the GQDs; TEM image (C) and UV−vis absorption (D) of the CdTe QDs.Inset: photocurrent responses of (a) ZnO/Au-PWE, (b) CdTe/ZnO/Au-PWE.

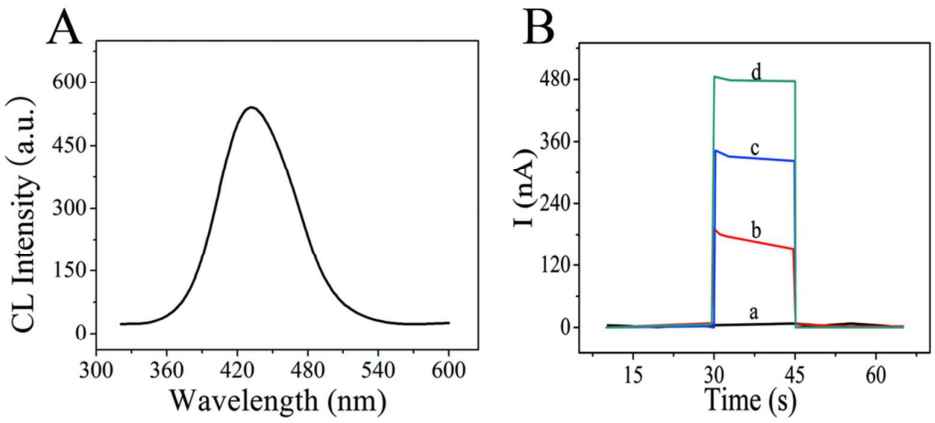

Figure S3. (A) CL spectra of luminol-HRP-H_2O_2 system.(B)Photocurrent responses of (a) ZnO/Au-PWE, (b) CdTe/Au-PWE, (c) CdTe/ZnO/Au-PWE,(d) MSNs/CdTe/ZnO/Au-PWE

Electrophoresis analysis

For performance of the polyacrylamide gel electrophoresis, aliquots of 15 μL of each sample containing 15% bromophenol were loaded onto polyacrylamide gel and electrophoresed at 100V for 90 min at room temperature. The photograph was taken by BIO-RAD Molecular Imager.

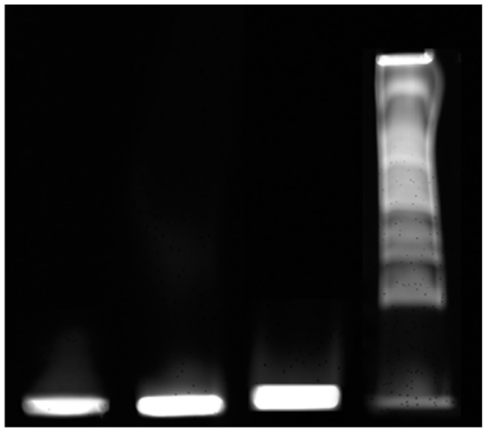

Figure S4A. Electrophoretic analysis of the HCR products..

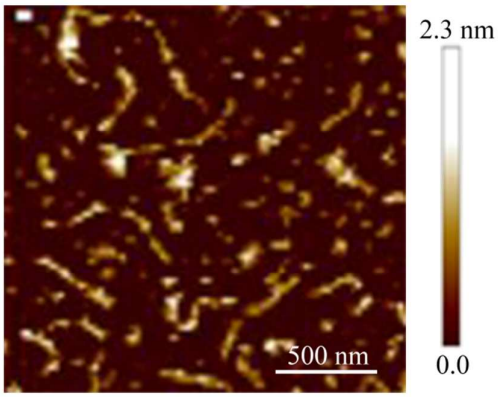

Figure S4B. AFM images of HCR products.

Optimization of the conditions

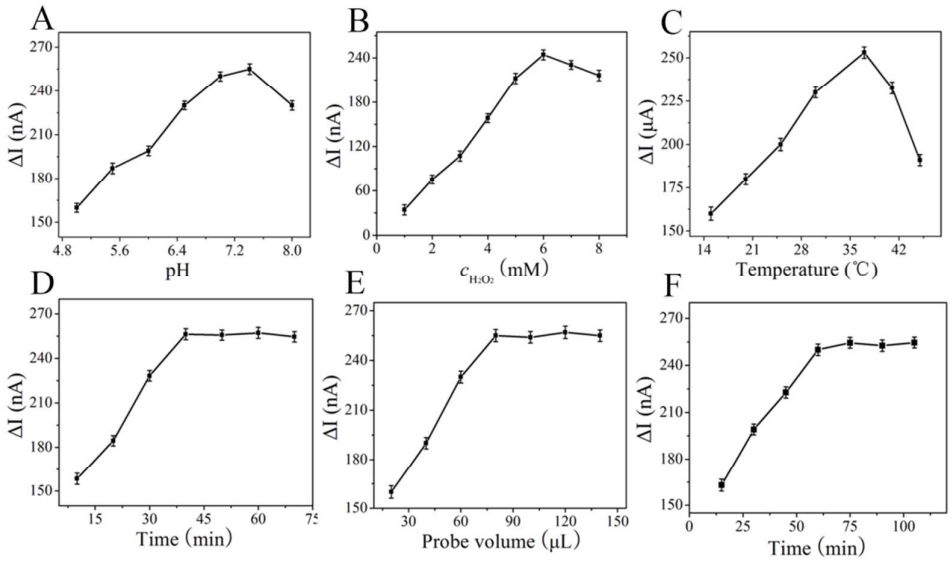

Figure S5. Optimization of (A) pH of the 0.1 M PBS, (B) H_2O_2 in 0.1 M PBS, (C) Incubation temperature, (D) Cell incubation time, (E) Probe volumes of the PEC biosensor, (F) Probe incubation time. The concentration of MCF-7 cells: 1.0×10^5cells·mL^{-1}.

To optimize the experimental condition for MCF-7 cells and N-glycan expression measurement, the effects of pH and temperature of the biosensor, concentration of H_2O_2, incubation time for the cells and GQDs@Con A were investigated. In addition, the amount of the GQDs@Con A was researched as well. A series of experiments were conducted to select optimal analytical conditions using the MCF-7 cell with concentration of 1×10^5 cells mL^{-1}.

The pH of the system is an important parament for the detection of the cell and N-glycan expression. We can see from the Figure S5A, when the pH was 7.4, the change of photocurrent intensity (ΔI) of the biosensor is the best compared with other conditons. So the experiments were carried out at pH with 7.4.

H_2O_2 was not only used as the oxidant in the luminol-HRP-H_2O_2 CL system, but also as the electron donors to scavenge photogenerated holes in valence band of CdTe QDs as well as to facilitate the generation of stable photocurrent. As can be seen in the Figure S5B, when the concentration of H_2O_2 was lower than 6.0 mM, the ΔI of the biosensor increased with increasing H_2O_2 concentration. However, when the concentration of H_2O_2 was further elevated, the ΔI of the biosensor would decrease with increasing concentration of H_2O_2. That may attribute to the oxidation of the CdTe QDs by excess H_2O_2 that yielded surface defects and traps. Therefore, 6.0 mM H_2O_2 was used in the experiments.

The temperature of the biosensor also greatly affected the sensitivity of the biosensor. Apparently, 37 ˚C, close to the normal temperature of the human body, is favorable for the assay (Figure S5C). So all the experiments were carried out at 37 ˚C ensuring the sensitivity of the biosensor.

In addition, incubation time of modified electrode for capturing MCF-7 cells and specifically recognizing GQDs@Con A were further optimized (Figure S5D and S5F). With the incubation time increased, more cells and GQDs@Con A conjugates were immobilized on the electrode. The ΔI of the biosensor increased because of the huge steric effect of cellls and GQDs@Con A s as well as competitively absorption of the GQDs to the CL. The ΔI increased and tended to a platform at 70 and 60 min respectivily, which were chosen as the optimal incubation times. Furthermore, the amount of the GQDs@Con A was investigated as well (Figure S5E). The ΔI increased

rapidly with the increase of the amount up to 80 μL, and then tended to be constant. Therefore 80 μL of the GQDs@Con A conjugates solution was employed in the experiment.

Specificity and stability of the biosensor

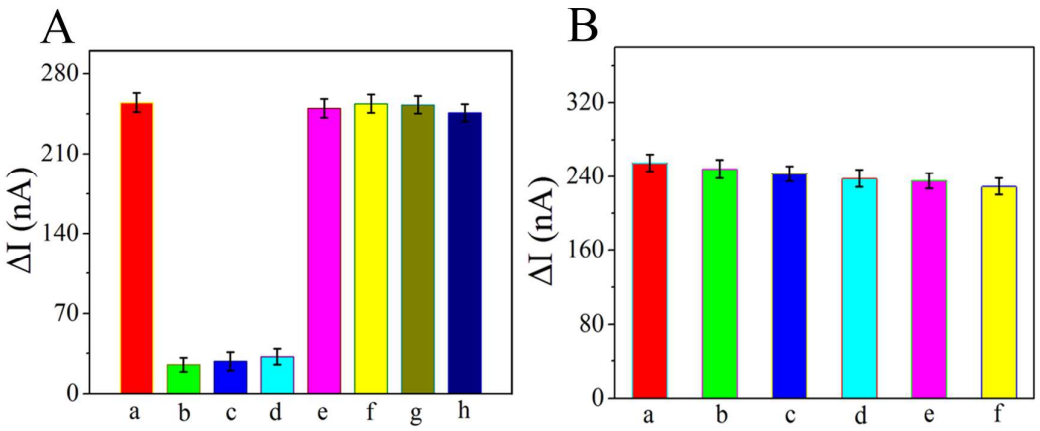

Figure S6. (A) Photocurrent responses of the immunoassay for $1x10^5$ cells mL^{-1} (a) MCF-7, (b) K562, (c) A549, (d) Hela, (e) MCF-7 + K562, (f) MCF-7 + A549, (g) MCF-7 + Hela, (h) MCF-7+ K562 + A549 + Hela. The error bars showed the standard deviation of five replicate measurements; (B) Stability of the as-prepared electrodes stored for (a) 5, (b) 10, (c) 15, (d) 20, (e) 25, (f) 30 days (stored in a humid environment at 4 ˚C).

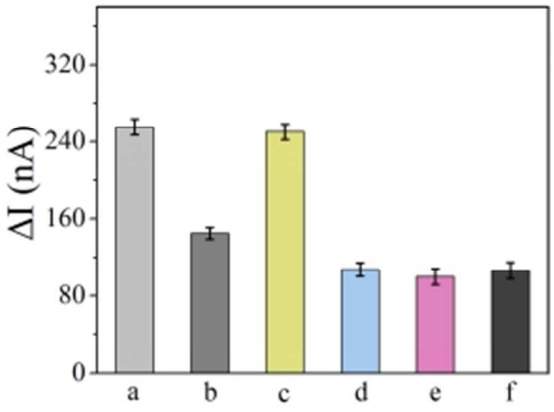

Figure S7. N-Glycan expression of MCF-7 cells surface without or with treatment of PNGase F for 24 h and after incubation with TM (1.2 μM) and BG (500 μM) for 48 h: (a) no treatment, (b) TM, (c) BG, (d) PNGase-F, (e)TM + PNGase-F, (f) BG + PNGase-F. The concentration of MCF-7 cell: 1.0×10^5 cells·mL^{-1}.

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