Electronic Supplementary Information

# A near-infrared light-controlled, ultrasensitive one-Step photoelectrochemical detection of dual cell Aapoptosis indicators on living cancer cells

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## EXPERIMENTAL METHODS

### Chemicals and Materials.

The ATP1A1 antibody (ATP1A1 Ab) was obtained from Proteintech Group (Chicago, IL, USA). The Annexin V antibody was purchased from Invitrogen Life Technologies Inc. (Carlsbad, CA, USA). Cetyltrimethylammonium bromide (CTAB), silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$) and ascorbic acid (AA) were purchased from ChengDu KeLong Chemical Co. Ltd. (Chengdu, China). 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), n-hydroxysulfosuccinimide (NHS), gold chloride ($HAuCl_4$), bovine serum albumin (BSA) and 6-mercaptohexanol (MCH) were obtained from Shanghai fine chemical materials institute (Shanghai, China). Carboxyl-terminated polyamidoamine dendrimer (PAMAM, fifth generation), doxorubicin (Dox), paraformaldehyde, 4',6-diamidino-2-phenylindole (DAPI) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The phosphate buffer solution (PBS, 0.1 M) was purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). Ferricyanide/ferrocyanide mixed solution ($[Fe(CN)_6]^{3-/4-}$, 5.0 mM) was prepared by dissolving potassium ferricyanide and potassium ferrocyanide with PBS (pH 7.4) solution.

The DNA oligonucleotides used in the experiment were synthetized from Shanghai Sangon

Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences of the DNA oligonucleotides were listed as following:

**Table S1.** The sequences used in the experiment.

| Name | Sequence (5'→3') |
|---|---|
| Primer | $NH_2$-TTT TTC CTA GAG CTA AGC TAT GAC CTG GT |
| Hairpin DNA 1 (H1) | GCT AAG CTA TGA CCT GGT CCA TGA CGA CCT TGA CCA GGT CAT AGC TTA GCT CTA GGC CAT GAC G |
| Hairpin DNA 2 (H2) | GCT AAG CTC AAG GTC GTC ATG GAC CAG GTC ATC CTA GCT AAG CTA TGA CCT GGT CCA TGA CG |
| Cy3 labeled hairpin DNA 1 (H1-Cy3) | Cy3- GCT AAG CTA TGA CCT GGT CCA TGA CGA CCT TGA CCA GGT CAT AGC TTA GCT CTA GGC CAT GAC G |
| Cy3 labeled hairpin DNA 2 (H2-Cy3) | Cy3- GCT AAG CTC AAG GTC GTC ATG GAC CAG GTC ATC CTA GCT AAG CTA TGA CCT GGT CCA TGA CG |

**Apparatus.**

The PEC measurements were recorded with a PEC workstation (Ivium, Netherlands) using a three-electrode system, in which a platinum wire was served as counter electrode, a saturated calomel electrode (SCE) was served as reference electrode and a bare or modified glassy carbon electrode (GCE, 4 mm diameter) was served as working electrode. Cyclic voltammetry (CV) and electrochemical deposition were performed with CHI 660e electrochemical workstation (Shanghai Chenhua Instrument, China). The photophysical characterizations of the synthesized nanomaterials were carried out by a UV-2550 spectrophotometer. The characterizations for the morphologies of the synthesized nanomaterials were performed by scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and high-resolution transmission electron microscopy (HRTEM, JEM-2100, JEOL, Japan). The laser confocal microscopy measurement was carried out by laser confocal microscope (Leica Onc., Heidelbery, Germany). The flow cytometry analysis was performed by a NovoCyte™ flow cytometry instrument (ACEA Biosciences, Inc., San Diego, CA, USA). Absorbance reading in MTT assay was performed using multiscan spectrum microplate reader (Thermo Electron Corp., NC, USA).

**Cell Culture.** The human breast cancer cell line (MDA-MB-231) was purchased from the Cell bank of type culture collection of the Chinese Academy of Science (Shanghai, China). The cells were grown in a 37 °C humidified atmosphere containing 5% $CO_2$, using Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 100 U/mL streptomycin and 100 U/mL penifcillin.

**Flow Cytometry Analysis.** The cells were plated on a 6-well plate at $1\times10^5$ cells per well and allowed to adhere overnight. The lifted cells were removed by washing with PBS solution for several

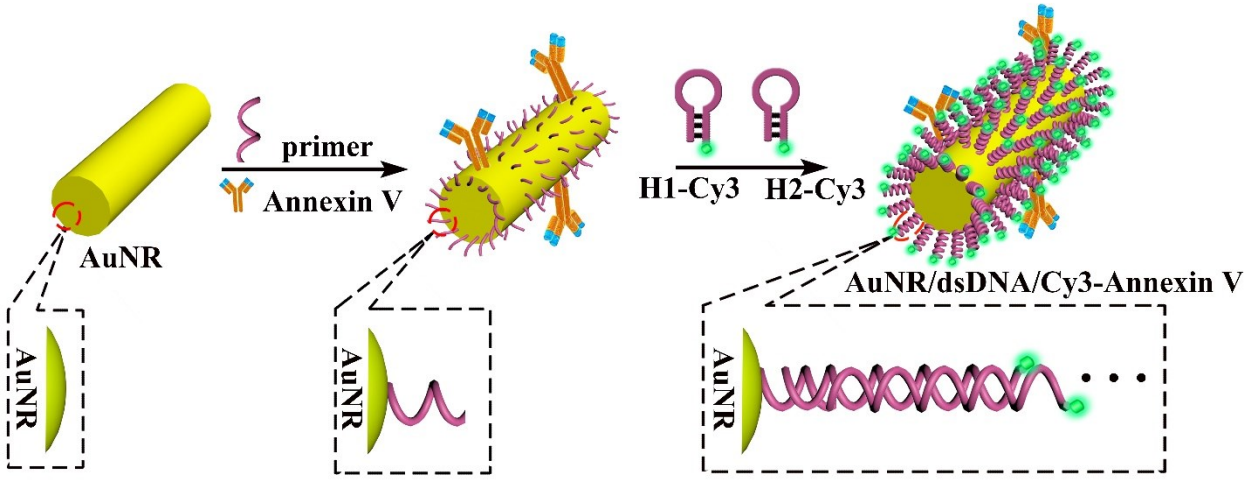
times. Then the fresh medium and drug solution were added, followed by gently mixing. The cells were incubated at 37 ℃ overnight. Subsequently, the cells were removed by trypsinization and washed gently for several times with PBS solution. The obtained cells were dispersed in 100 mL binding solution followed by addition of 5 mL Annexin V-fluorescein isothiocyanate and 5 mL propidium iodide solution in the dark. Flow cytometry analysis was conducted on a NovoCyte™ (ACEA Biosciences, Inc., San Diego, CA, USA) by counting 20000 events, and the data were analyzed by FlowJo 7.6.5 software.

**Preparation for the AuNR/MB-dsDNA/Annexin V Bioconjugates ($Tag_{kinetic}$).** The preparation of AuNRs was according to the literature with minor modifications. Firstly, 5 mL CTAB solution (0.2 M) was mixed with 5 mL $HAuCl_4$ solution (0.5 mM). Subsequently, 0.6 mL ice-cold $NaBH_4$ solution (10 mM) was added. Under vigorous stirring, the color of the mixture solution was slowly changed from yellow to brown. Then the solution was stirred for 3 h to obtain the seed solution for preparation of AuNRs in the next step. To synthesize the AuNRs, firstly, 1.5 mL $AgNO_3$ solution (4 mM) was mixed with 50 mL $HAuCl_4$ solution (1 mM). (The silver ions in $AgNO_3$ solution was very necessary to control the dimensions of AuNRs in growth solution.) Then, 50 mL CTAB solution (0.2 M) and 0.7 mL AA solution was added to the mixture solution. Under gently stirring, 120 μL the seed solution was dropped to the mixture to initiate the growth of AuNRs. Then the obtained solution was kept constant for 24 h in room temperature. The obtained AuNRs solution was centrifuged and washed for several times to remove the redundant reagents. Finally, the product was dispersed in 2 mL ultrapure water and stored in 4 °C for further usage.

For preparation of the AuNR/MB-dsDNA/Annexin V bioconjugates, briefly, 1 mL the prepared AuNR solution was mixed with 50 μL Annexin V solution and 10 μL primer solution (100 μM),

followed by stirring at 4 °C for 16 h. Then, 50 μL H1 (100 μM) and 50 μL H2 (100 μM) were added to the mixture and stirred at 37 °C for 4 h. After that, 100 μL MCH solution was dropped to the solution and stirred for 40 min to block the nonspecific binding sites on the AuNR. Finally, 50 μL MB solution (1 mg/mL) was dropped to the mixture under vigorously stirring for 2 h. The obtained solution was centrifuged and washed for several times to remove the redundant reagents.

**Preparation for the AuNR/Cy3-dsDNA/Annexin V Bioconjugates.** As shown in Scheme S1, to prepare the AuNR/Cy3-dsDNA/Annexin V bioconjugates, briefly, 1 mL AuNR solution was mixed with 50 μL Annexin V solution and 10 μL primer solution (100 μM), followed by stirring at 4 °C for 16 h. Then, 50 μL H1-Cy3 (100 μM) and 50 μL H2-Cy3 (100 μM) solution were added to the mixture and stirred at 37 °C for 4 h. Subsequently, 100 μL MCH solution was dropped to the solution and stirred for 40 min to block the nonspecific binding sites on the AuNR. The obtained solution was centrifuged and washed for several times to remove the redundant reagents.

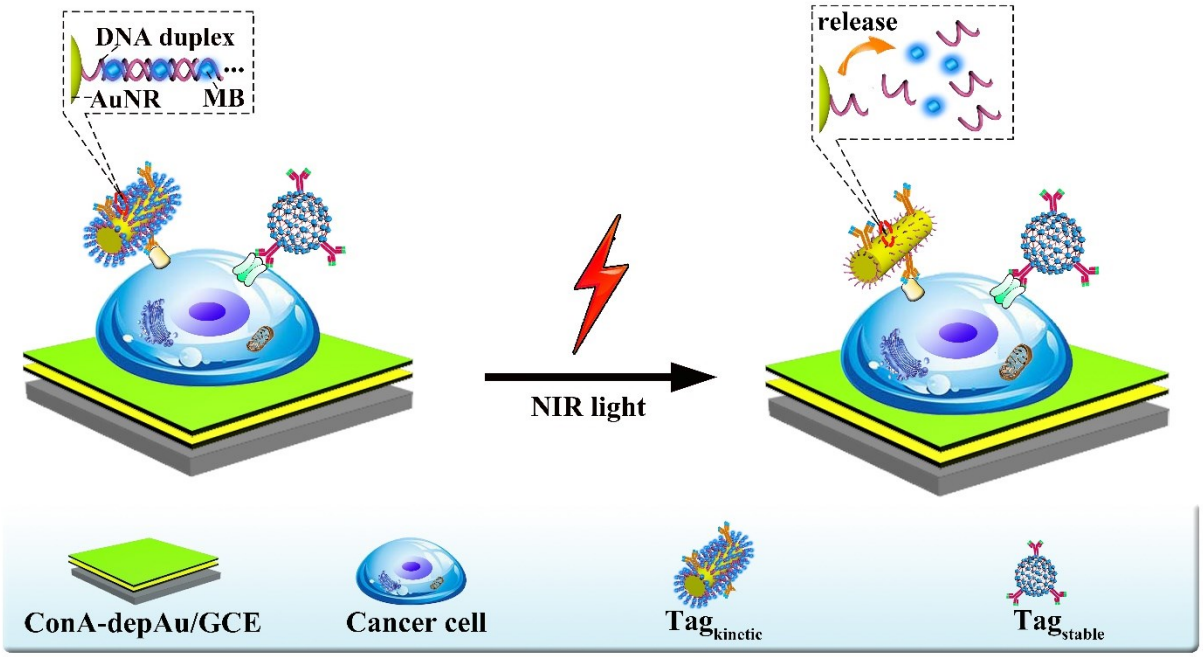



**Scheme S1.** Preparation procedure for the AuNR/Cy3-dsDNA/Annexin V bioconjugates.

**Preparation for the CQDs/PAMAM/ATP1A1 Ab Bionanoconjugates ($Tag_{stable}$).** The preparation of CQDs was according to the literature.[1] To prepare CQDs/PAMAM/ATP1A1 Ab bionanoconjugates, briefly, 500 μL PAMAM solution was mixed with EDC (0.2 M) and NHS (0.05

M) for 20 min at room temperature to active the carboxyl groups on PAMAM. Then the obtained solution was mixed with 50 μL ATP1A1 Ab solution (5 mg/mL) and 300 μL CQDs solution and gentle stirred for 2 h. The obtained CQDs/PAMAM/ATP1A1 Ab bionanoconjugates was washed and centrifuged for several times to remove the unbound reagents. The prepared CQDs/PAMAM/ATP1A1 Ab was redispersed in PBS with 100 μL MCH for 2 h to block the nonspecific sites.

**Fabrication of the PEC Cytosensor.** The schematic illustration for PEC cytosensor was demonstrated in Scheme S2. Before modification, the GCE ($\Phi$ = 4 mm) was polished by 0.3 μm alumina slurry, followed with ultrasonicating in ultrapure water for several times to obtain a mirror-like surface. Afterward, the electrode was immersed in 1 % $HAuCl_4$ solution for electrochemical deposition to modify gold nanoparticle layer (depAu) on the electrode with the constant potential of -0.2 V for 30 s. Subsequently, the depAu modified electrode was incubated with 20 μL Con A solution (1 mg/mL) for 2 h to absorb Con A on the modified electrode. Finally, the Con A/ AuNPs modified electrode was immersed with 20 μL BSA solution (1 mM) to block the nonspecific binding sites on the electrode. After each step, the electrode was washed by ultrapure water to remove the redundant reagents.




**Scheme S2.** Schematic illustration for PEC cytosensor.

**PEC Measurement.** The PEC measurement was carried on a sandwich-type analytical procedure. Before measurement, the cytosensor was incubated with cells dispersed in culture medium at 37 °C for 40 min to capture cells on the cytosensor. Subsequently, the obtained cytosensor was incubated with 15 μL $Tag_{kinetic}$ solution and 15 μL $Tag_{stable}$ solution at 37 °C for 40 min. Finally, the PEC measurement of the cytosensor was investigated with a PEC workstation in PBS (0.1 M, pH 7.0) solution containing 0.1 M AA. A NIR light source was provided during the PEC measurement with a bias potential of 0 V.

**REFERENCES**

1 S. Y. Lim, W. Shen, Z. Q. Gao, *Chem. Soc. Rev.*, 2015, **44**, 362-381.

## RESULTS AND DISCUSSION

**Electrochemical Characterization of the Sensing Interface.** To confirm the fabrication process of the sensing interface, cyclic voltammogram (CV) characterization was employed to record the stepwise modification process in 0.1 M PBS (pH 7.0) containing 5 mM $[Fe(CN)_6]^{3-/4-}$. In Figure S1A, the bare glassy carbon electrode (GCE) presented a pair of redox peak for $[Fe(CN)_6]^{3-/4-}$ (curve a). After depAu was deposited on the sensing interface, the redox peak obviously increased (curve b) owing to the excellent conductivity of depAu. Subsequently, the redox peak decreased (curve c) when the Con A was modified on the electrode surface *via* Au-N covalent bond to capture the living cells. Finally, the redox peak furtherly decreased (curve d) after the modified sensing interface was blocked with HT solution. The reason for these could be attributed to the fact that Con A and HT could hinder the electron transfer. Furtherly, the morphology characterization of depAu was recorded by SEM. As shown in Figure S1B, the depAu demonstrated a large surface area with a flower-like structure (inset of Figure S1B) to promote the electron transfer. These data demonstrated that the sensing interface was modified successfully.

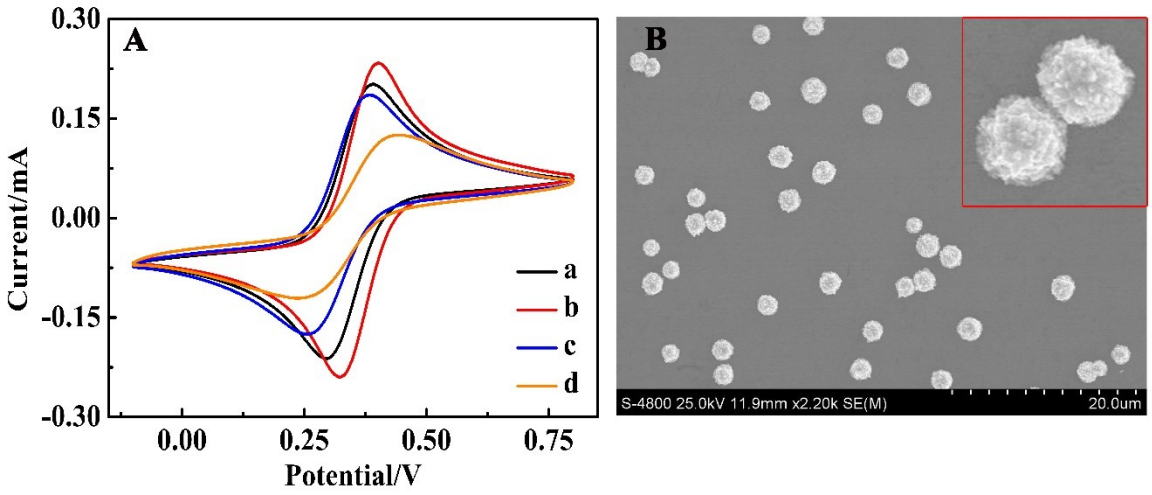



**Figure S1**. (A) CV characterizations for the sensing interface at (a) GCE, (b) depAu/GCE, (c) Con A/ depAu/GCE, (d) HT/ Con A/ depAu/GCE immersed in 5 mM $[Fe(CN)_6]^{3-/4-}$ electrolyte at 0.05 V/s scan rate. (B) SEM characterization for depAu. Inset of Figure S1B was a partially enlarged

SEM image of depAu.

**Mechanism of Mixed Signal Decryption Strategy.** For a kinetic controlled mixed signal curve decryption strategy for dual-analytes detection, it is necessary to construct a relationship equation between the detected mixed signal and the quantity of different targets. Normally, the detected mixed photocurrent signal output was contributed to each individual photocurrent from different signal labels, which were positive correlation with quantity of their corresponding targets. The relationship equation could be demonstrated as:

$$I = \sum I_{signal\ tag_i} \qquad (1)$$

in which $I$ and $I_{signal\ tag_i}$ represent the detected photocurrent value and photocurrent value produced from individual signal tags. In this work, a relationship equation between the detected mixed signal ($I_t$) and the signals produced from different signal tags ($I_{CQD_t}\ and\ I_{MB_t}$) at the time of $t$ could be demonstrated as:

$$I_t = I_{CQD_t} + I_{MB_t} \qquad (2)$$

For the signal response of the signal tag CQD, the calibration curve was plotted as:

$$I_{CQD_t} = mc_{CQD} + n \qquad (3)$$

in which m and n are the variables obtained with a least-squares linear regression, $c_{CQD}$ was the concentration of CQD. For the signal response of MB, the calibration curve was plotted as:

$$I_{MB_t} = ac_{MB_t} + b \qquad (4)$$

in which a and b are the variables obtained with a least-squares linear regression, $c_{MB_t}$ was the concentration of MB at the time of $t$. The $Tag_{kinetic}$ was constructed by anchoring photoactive signal label MB embed DNA double helix on AuNR, which could convert a near-infrared light (NIR) energy to heat energy for denaturation of DNA double helix to release the MB, leading to a kinetically reduced photocurrent signal. The kinetically release process of MB could be demonstrated as:

$$MB_{AuNR} \leftrightharpoons MB_{released}$$

in which the $MB_{AuNR}$ demonstrated the MB embed in DNA double helix on AuNR, and the $MB_{released}$ represented the released MB under NIR irradiation. The corresponding reaction rate equation could be demonstrated as:

$$ln(c_{MB}/c_{MB_t}) = kt \quad (5)$$

in which the $c_{MB}$, $c_{MB_t}$ and k demonstrated the initial concentration of MB embed in DNA double helix on AuNR, concentration of MB embed in DNA double helix on AuNR after irradiation time of *t* and the reaction constant, respectively. Therefore, the concentration of MB at the irradiation time of *t* could be demonstrated as:

$$c_{MB_t} = c_{MB}/e^{kt} \quad (6)$$

Thus, according to the eq (3) and eq (6), the photocurrent produced from MB at the irradiation time of *t* could be demonstrated as:

$$I_{MB_t} = ac_{MB}/e^{kt} + b \quad (7)$$

Herein, according to the eq (2), eq (4) and eq (7), the detected photocurrent signal at the irradiation time of *t* could be demonstrated as:

$$I_t = (a/e^{kt})c_{MB} + mc_{CQD} + n + b \quad (8)$$

in which the $a/e^{kt}$, m, n and b were constants at a certain irradiation time. Thus, it was possible to calculate $c_{MB}$ and $c_{CQD}$ based on two photocurrent at two different irradiation times in one-step detection for dual-analytes detection.

**Preparation procedure for the CQDs/PAMAM-ATP1A1 bio-nanoconjugates.** The Tagstable was constructed by anchoring antibody of Sat (ATP1A1 Ab) on the CQDs-PAMAM nanoconjugates (Figure S2A). To characterize the Tagstable (CQDs/PAMAM-ATP1A1 Ab bio-nanoconjugates), we

use high resolution transmission electron microscopy (HRTEM) and Scanning Electron Microscope (SEM) to characterize the average particle size and morphology of CQDs and CQDs-PAMAM nanoconjugates, respectively. In Figure S2B and C, the CQDs and CQDs-PAMAM nanoconjugates displayed average distribution around 5 nm and 100 nm in diameter, respectively. Besides, the water soluability and photophysical properities of CQDs-PAMAM nanoconjugates were tested with ambient daylight and ultraviolet light, respectively. As shown in the inset image of Figure S2C, the CQDs- PAMAM nanoconjugates showed a yellow color under ambient datlight, while a blue color under ultraviolet light, indicating that the synthesized materials possessed an excellent dispersity and fluorescence property. After morphology and photophtsical characterizations, we furtherly investigated the photocurrent response of the CQDs-PAMAM nanoconjugates on sensing interface. Different from the phot-induced kinetic release process of MB in AuNR-dsDNA-MB nanoconjugate, the photocurrent of CQDs-PAMAM nanoconjugates should be a stable signal since there were no phot-induced kinetic release process occurred in CQDs-PAMAM nanoconjugates. As shown in Figure S5, under a sustaining NIR light irradiation, the CQDs-PAMAM nanoconjugates performed a stable and constant photocurrent response, which was in accordance with our anticipation.

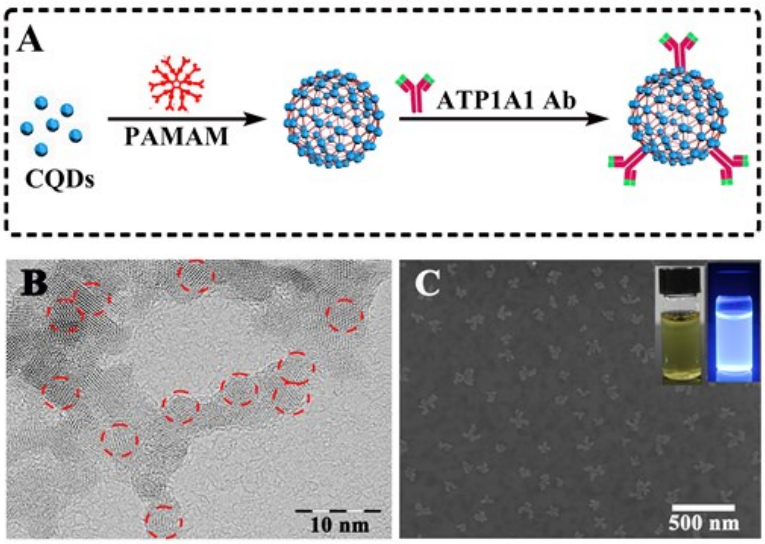


**Figure S2.** (A) Preparation procedure for the CQDs/PAMAM-ATP1A1 bio-nanoconjugates; (B) high resolution transmission electron microscopy (HRTEM) image of the CQDs; (C) SEM image of the CQDs-PAMAM. Insets of (C) shows images for the CQDs/PAMAM solutions under an ambient daylight (left) and ultraviolet light (right), respectively.

Furtherly, to monitor the NIR light controlled release process and to quantitatively measure the release level, the Tagkinetic (AuNR/MB-dsDNA/Annexin V bioconjugates) have been explored on PEC cytosensor to record photocurrent response. Apoptotic MDA-MB-231 cancer cells treated by Dox with different concentrations (1, 5, 20, 50, 100, 150, 200 μg/mL) were immobilized on the sensing interface by concanavalin (Con A) to furtherly bind with the AuNR/Cy3-dsDNA/Annexin V bioconjugates. As can be seen from Figure 1D, when a NIR light was given, an obviously initial photocurrent response could be observed from the Tagkinetic bound on target Pho. Then the photocurrent response gradually decreased with increasing the light irradiation time, which could be explained by the fact that the AuNR could convert the NIR energy to heat energy for denaturation of dsDNA to release the signal label MB, leading to a kinetically reduced photocurrent signal. As a contrast, the photocurrent of MB without kinetic release process was presented in Figure S6, which presented a stable photocurrent response. Subsequently, the relationship between NIR light controlled release process and the concentration of drug treated cancer cells were investigated using a PEC method. As can be seen from Figure 1D and E, the initial photocurrent increased with increasing the drug concentration, which could be attributed to the fact that with increasing the drug concentration, the apoptotic level of cells increased, leading to more binding sites to bind with the signal materials AuNR/MB-dsDNA/Annexin V bioconjugates. And the linear relationship was Iinitial photocurrent = 3.45cdrug + 117.33, and a correlation coefficient of 0.9746 (Figure 1E), which

demonstrated that the Tagkinetic can be applied for quantitative determination of drug concentration.

## FIGURES

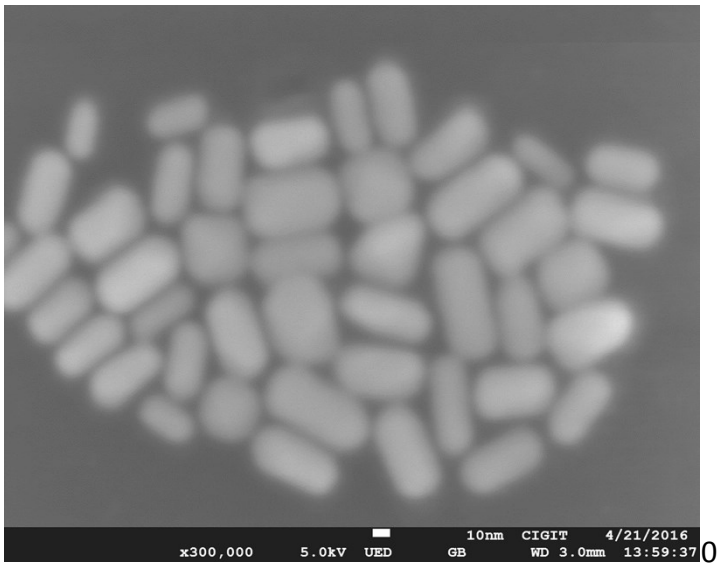



**Figure S3.** SEM image of the AuNR.

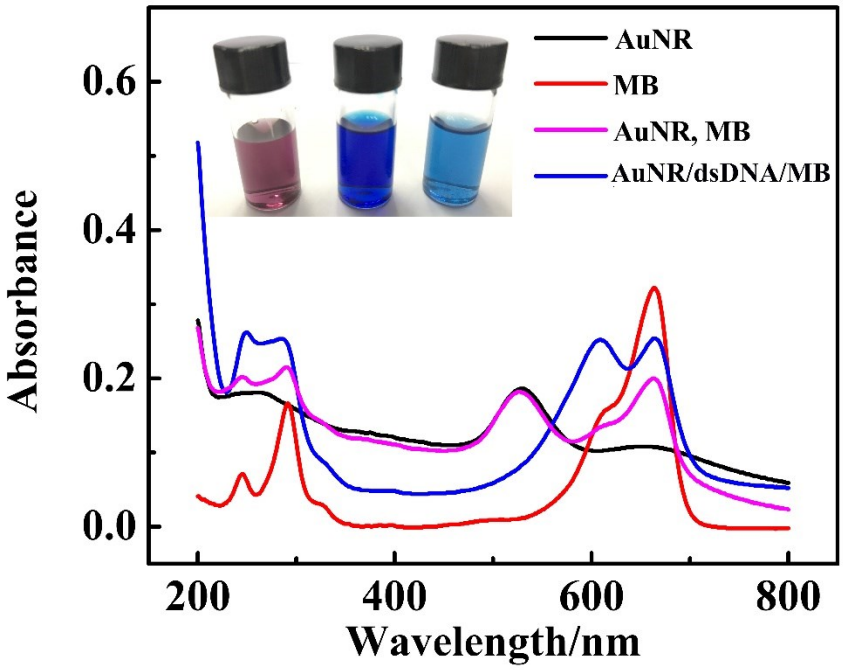



**Figure S4.** UV-vis adsorption spectra characterization for AuNR; MB; AuNR and MB; AuNR/dsDNA/MB (inset from left to right, images for the AuNR; MB and AuNR/dsDNA/MB solutions, respectively).

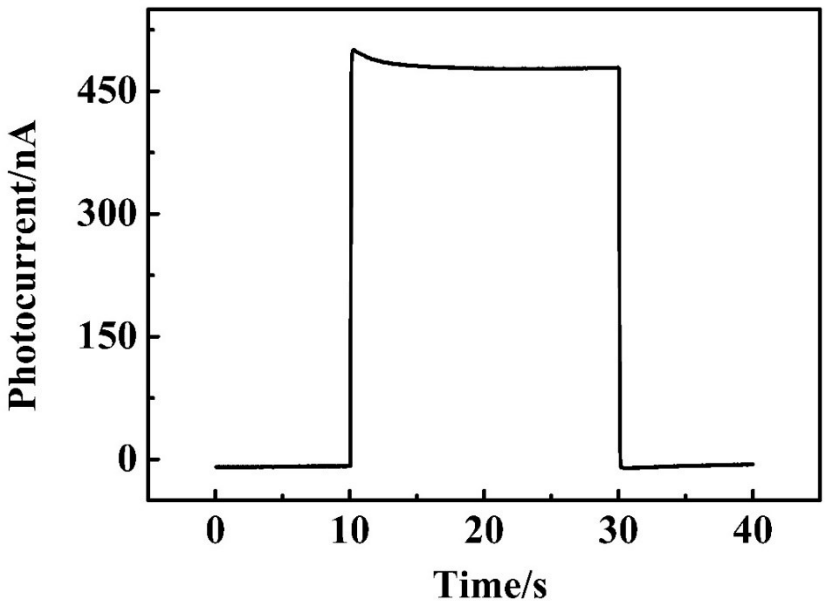



**Figure S5.** Photocurrent response of the CQDs-PAMAM under a NIR irradiation in PBS solution containing 0.1 M AA.

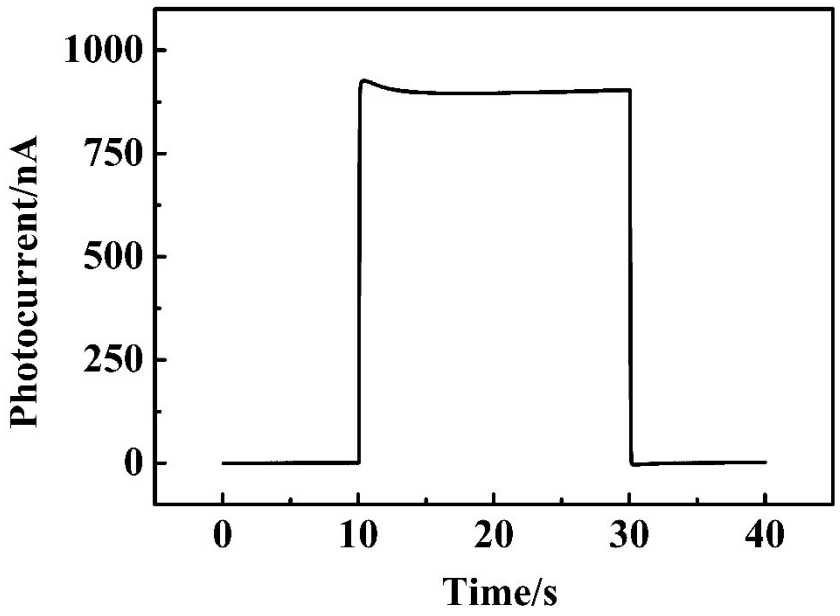



**Figure S6.** Photocurrent response of the MB stably immobilized on sensing interface under a NIR irradiation in PBS solution containing 0.1 M AA.

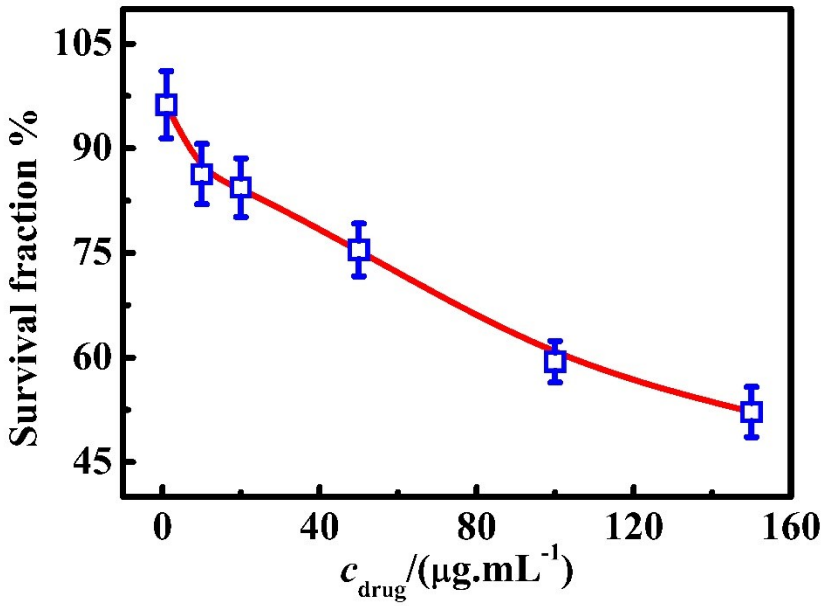



**Figure S7.** MTT results of MDA-MB-231 cancer cells treated with 1, 10, 20, 50, 100, 150 μg/mL Dox, respectively.

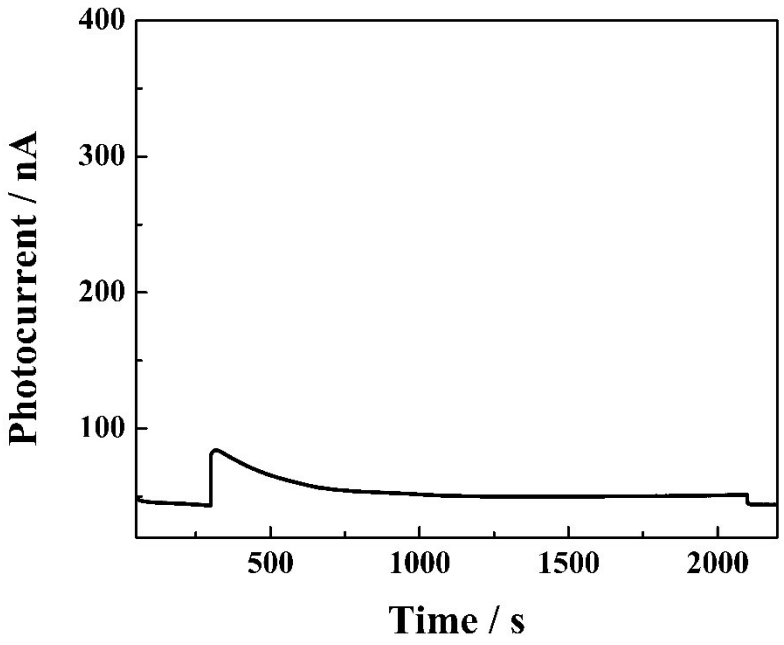



**Figure S8.** The background photocurrent response of the proposed PEC biosensor.

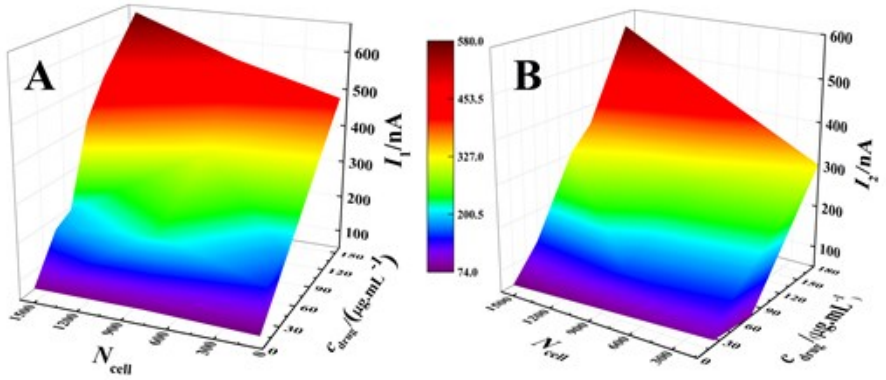



**Figure S9**. Photocurrent responses of the proposed PEC assay at irradiation time of 150 s (A) and 200 s (B) for detection of total cell numbers and drug cencentrations with different total number of cells range from 20 to 1500, and different concentration of drugs range from 1 □g/mL to 150 □g/mL.

**Authors Contributions**

Yingning Zheng, Wenbin Liang and Ruo Yuan conceived the project. Yingning Zheng performed the experiments and analyzed the data. Chengyi Xiong performed the experiments and assisted to revise the paper. Ruo Yuan and Wenbin Liang supervised the project. Yingning Zheng, Ruo Yuan, Yaqin Chai, Wenbin Liang and Ying Zhuo commented and revised the paper.