Electronic supplement Information (ESI)

A Label-free Multiplex Electrochemical Biosensor for Detection of Three Breast Cancer Biomarker Proteins Employing Dye/Metal Ions-loaded and Antibodies-Conjugated Polyethyleneimine-Gold Nanoparticles

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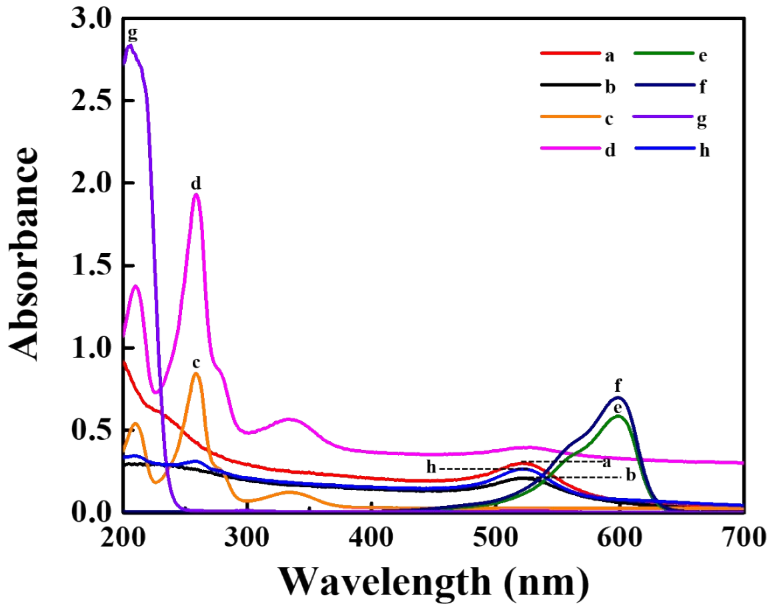

Fig. S1 UV-Visible absorption spectra of (a) PEI-AuNPs, (b) antibody-PEI-AuNPs, (c) AQ, (d) AQ-antibody-PEI-AuNPs, (e) TH, (f) TH-antibody-PEI-AuNPs, (g) Ag^{+}, and (h) Ag^{+}-antibody-PEI-AuNPs.

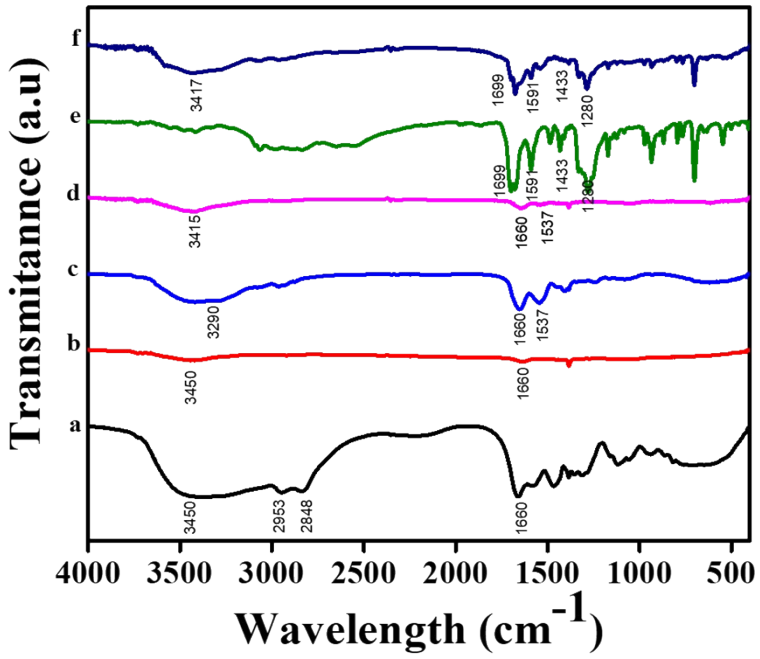

Fig. S2 FT-IR spectra of PEI (a), PEI-AuNPs (b), antibody (c), antibody-PEI-AuNPs (d), AQ (e), and AQ-antibody-PEI-AuNPs (d).

The FT-IR spectra of PEI (a) shows characteristic peaks at 3,450 and 1,660 cm^{-1}, which can be assigned to stretching and bending vibrations of N-H and -NH_2, respectively. The peaks at 2953 and 2848 cm^{-1} can be assigned to C-H stretching. The PEI-functionalized AuNPs exhibit the small characteristic absorption peaks of amine groups located at 3,450 and 1,660 cm^{-1} (b)**,** confirming the presence of PEI in the PEI-AuNPs product. After the immobilization of antibody onto the surface of PEI-AuNPs, the absorption peak at 1660 cm^{-1} and 1537 cm^{-1} can indicate C=O, C-N stretching or N-H bending, which are associated with the antibody. Subsequently, when AQ is attached to antibodies-conjugated PEI-AuNPs, the observable peak at 1699 cm^{-1} is the C=O stretching vibration peak of AQ. Moreover, the feature bands at 1591 cm^{-1}, 1433 cm^{-1}, and 1280 cm^{-1} indicates the skeletal structure of aromatic ring, which is consistent with that of AQ (e). In addition, the peak at 3417 cm^{-1} belongs to N-H, C-H stretching, and OH bonding (f), which correspond to those of PEI, antibodies, and AQ (superimposing broad peak). It is plausible that AQ and antibodies has been successfully attached on PEI-AuNPs.

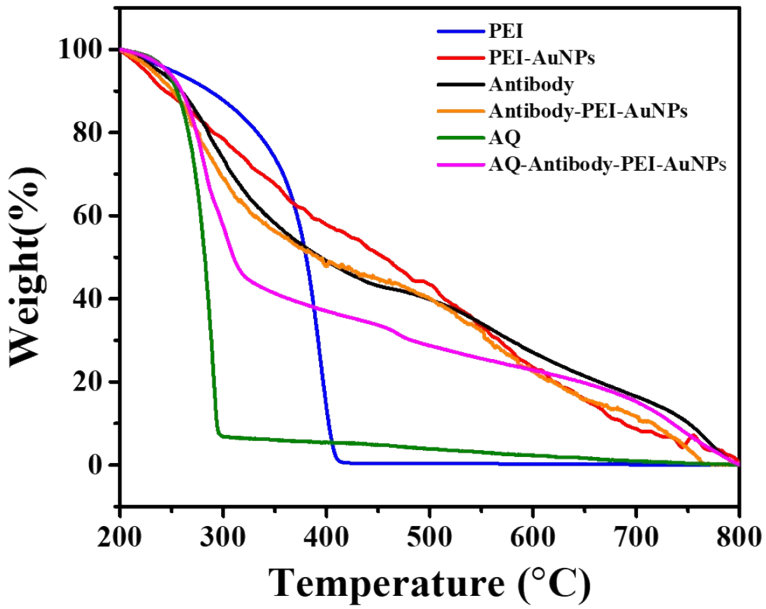

Fig. S3 TGA curves heating from 25 to 800 °C (10 °C min^{-1}) under a flow of N_2.

As seen in Fig. S3, PEI is completely degraded at *ca*. 420 ºC while AQ is completely degraded at *ca*. 290 ºC. PEI-AuNPs degrade continueously over the range 200-800 ºC and antibody degrade with three steps of 200-450, 450-700, and 700-800 ºC. Antibody-conjugated PEI-coated AuNPs present the three-step degradation profile similar to that of antibody, indicating successful attachment of antibody onto PEI-AuNPs. The final product, AQ/antibody-conjugated PEI-coated AuNPs, shows the deep first step degradation close to the degradation temperature of AQ, indicating the attachment of AQ onto antibody-conjugated PEI-AuNPs. Moreover, the second step degradation of the final product is similar to that of antibody, suggesting presence of antibody in the PEI-AuNPs. Since the PEI-AuNPs is degraded over wide range, the degradation profile of PEI-AuNPs is superimposed and might have no contribution to the character of degradation profiles of the products, antibody-PEI-AuNPs and AQ-antibody-PEI-AuNPs. This result agrees well with that of the FT-IR experiment.

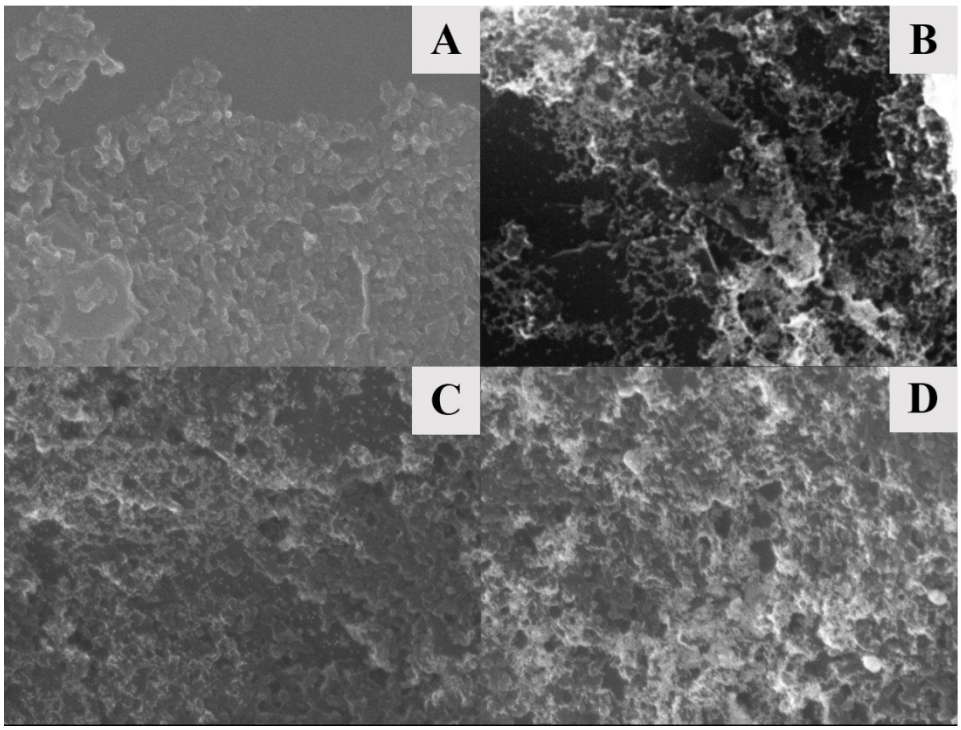

Fig. S4 SEM images of (A) bare SPCE and (B) anti-MUC1-AQ-PEI-AuNPs-, (C) anti-CA15-3-TH-PEI-AuNPs-, and (D) anti-HER2-Ag-PEI-AuNPs-modified SPCEs.

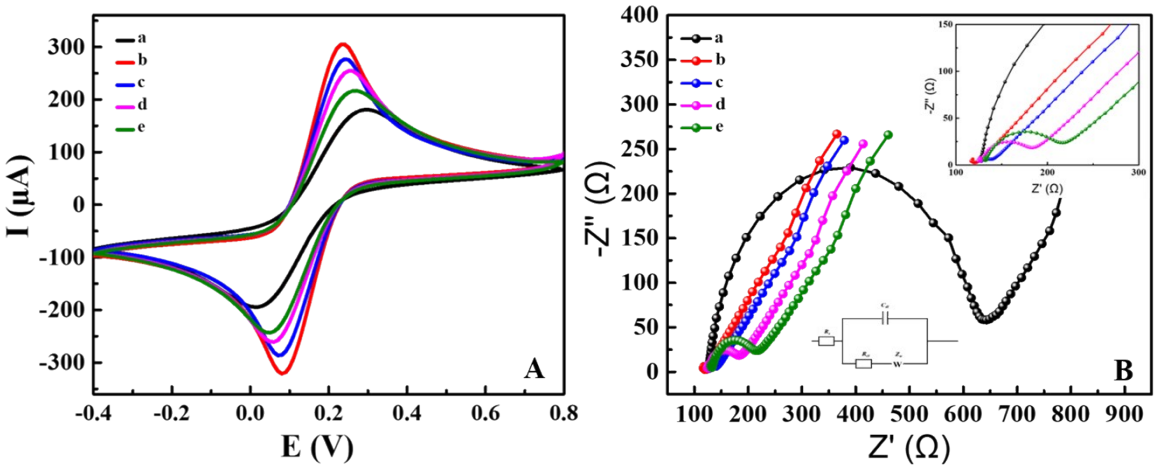

Fig. S5 The signal change of CV curves (A) and EIS spectra (B) during the fabrication process of the immunosensor, in 0.010 M PBS (pH 7.4) solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 50 mV/s for (a) bare SPCE, (b) PEI-AuNPs modified SPCE, (c) antibodies-PEI-AuNPs modified SPCE, and (d) antibodies-PEI-AuNPs modified SPCE after blocked with 0.05% BSA and (e) after incubation with antigens. (the inset shows the magnified area of low impedance and the equivalent circuit model)

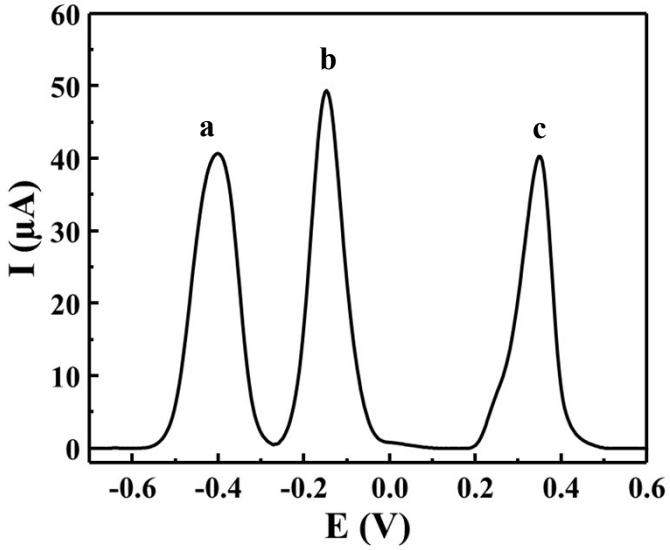

Fig. S6 Typical SWV signals of AQ-anti-MUC1-PEI-AuNPs (a), TH-anti-CA15-3-PEI-AuNPs (b), and Ag^{+}-anti-HER2-PEI-AuNPs (c)

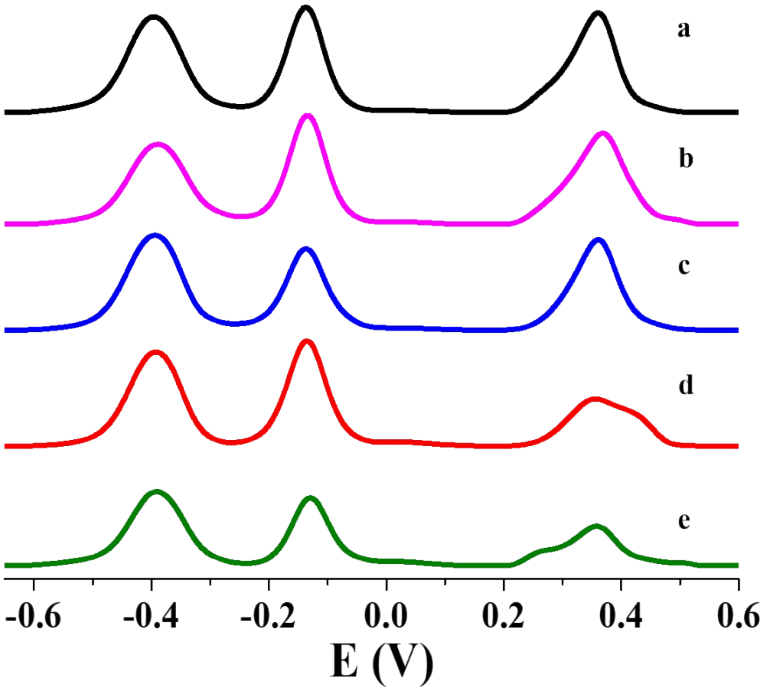

Fig. S7 SWV measurements for the investigation of cross-reactivity; blank (a) single analyte of 25 ng mL^{-1} MUC1 (b), 25 U mL^{-1} CA15-3 (c), and 25 ng mL^{-1} HER2 (d); the analyte mixture containing 25 ng mL^{-1} MUC1, 25 U mL^{-1} CA15-3 and 25 ng mL^{-1} HER2 (e).

Table S1 Comparison of the analytical performances of electrochemical immunosensors

Biomarker	Electrode modification	Detection	Linear range	LOD	Ref.
CA 15-3, HER2	AP-Anti1/Anti2/AuNPs/bi-SPCE (sandwich-type immunosensor)	LSV	CA 15-3: 0-70 U mL^{-1} HER2: 0-50 ng mL^{-1}	CA 15-3: 5.0 U mL^{-1} HER2: 2.9 ng mL^{-1}	[1]
HER2	Anti-HER2/Fe_3O_4NPs (label-free immunosensor)	DPV	0.01-10 ng mL^{-1} and 10-100 ng mL^{-1}	9.95x10^{-4} ng mL^{-1}	[2]
MUC1	AuNPs-graphite SPE by EIS AuNPs-gold SPE by DPV (label-free immunosensor)	EIS DPV	EIS: 2.5-15 ng mL^{-1} DPV: 0-10 ng mL^{-1}	EIS: 3.6 ng mL^{-1} DPV: 0.95 ng mL^{-1}	[3]
MUC1	APT/SA/AuNPs-GO-PEDOT/FTO (label-free immunosensor)	DPV	0.1 fg mL^{-1}-1 µg mL^{-1}	1 x10^{-6} ng mL^{-1}	[4]
CEA, CA15-3, CA125	Anti/MB-Chi/GR/ITO (label-free immunosensor)	LSV	CA15-3: 0.0001-0.0025 U mL^{-1} and 0.0025-0.1 U mL^{-1}	CA15-3: 0.00004 U mL^{-1}	[5]
MUC1, CA15-3, HER2	AQ/Anti-MUC1/PEI-AuNPs/SPCE TH/Anti-CA15-3/PEI-AuNPs/SPCE Ag^+/Anti-HER2/PEI-AuNPs/SPCE	SWV	MUC1: 0.10-100 ng mL^{-1} CA15-3: 0.10-100 U mL^{-1} HER2: 0.10-100 ng mL^{-1}	MUC1: 0.53 ng mL^{-1} CA15-3: 0.21 U mL^{-1} HER2: 0.50 ng mL^{-1}	This work

Alkaline phosphatease (AP), Gold nanoparticles (AuNPs), Antibody (Anti), Screen-printed carbon electrode (SPCE), Linear sweep voltammetry (LSV), Iron oxide nanoparticles (Fe_3O_4NPs), Differential pulse voltammetry (DPV), Electrochemical impedance spectroscopy (EIS), Screen printed electrode (SPE), Aptamer (APT), Streptavidin (SA), Graphene oxide (GO), Poly(3,4-ethylenedioxythiophene) (PEDOT), Fluorine tin oxide (FTO), Methylene blue-chitosan (MB-Chi), Graphene (GR), Indium tin oxide glass (ITO), Anthraquinone-2-carboxylic acid (AQ), Thionine chloride (TH), $AgNO_3$ (Ag^+), Anti-mucin1 (anti-MUC1), Anti-cancer antigen 15-3 (anti-CA15-3), Anti-human epidermal growth factor receptor 2 (anti-HER2), Polyethylenimine (PEI), Square wave voltammetry (SWV).

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Calculation of % Decreasing Current

The decreasing current percentage is measured before (I_0) and after (I) the immunoreaction between antibodies and antigens. The percentage of decreasing current is calculated as:

$$\% \text{ decreasing current} = \frac{(I_0 - I)}{I_0} \times 100$$