Electronic Supplementary Information (ESI)

Antibody-free detection of protein phosphorylation using intrinsic peroxidase-like activity of platinum/carbon dot hybrid nanoparticles

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Materials and chemicals

phytic acid (50%) in H_2O, Poly(ethylene glycol) (M_n=400), chloroplatinic acid hexahydrate (H_2PtCl_6•$6H_2O$), zirconyl chloride octahydrate ($ZrOCl_2$•$8H_2O$), poly(ethylene glycol) methyl ether thiol (SH-PEG, M_n=6000), Tris base, $MgCl_2$, NaCl, H_2O_2, 3,3',5,5'-tetramentylbenzidine(TMB), 3,3'-Diaminobenzidine (DAB), *o*-phenylenediamine (OPD), GF 109203X, CI-1033, adenosine tri-phosphate (ATP) were bought from Sigma-Aldrich. Dephosphorylated myelin basic protein (MBP), protein Kinase C alpha Human Recombinant was purchased from PROSPEC. PKC lipid activator was obtained from Millipore. The SKBR-3 and MCF-7 cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA). MCF-7 cells were grown in DMEM supplemented with 10% FBS. SKBR-3 cells were grown in McCoys 5A medium supplemented with 10% FBS. All of these breast cancer cell lines were incubated at 37 °C in a 5% CO2 atmosphere. Bioactive EGF was purchased from Shenandoah Biotechnology, Inc (Warwick, PA).

Instruments

The morphology and microstructure of samples were analyzed by transmission electron microscopy (TEM) on a JEM-2100 instrument. X-ray photoelectron spectra (XPS) were measured on a PHI-550 spectrometer by using MgKα radiation (hν=1253.6 eV) with a base vacuum operated at 300 W. The Fourier transform infrared (FTIR) spectra were measured on a Nicolet 360 FTIR spectrometer by using the KBr pellet technique. The UV-vis absorption spectrums were obtained with a Du-730 Life Science spectrophotometer (Beckman Coulter, USA). Colorimetric signals of the assays were quantified by a Synergy H4 microplate reader (BioTek, Winooski, VT)

Preparation of CDs

The CDs were synthesized by mixing 2 mL 50% phytic acid (PA) and 1 mL poly(ethylene glycol) (PEG-400) in 10 ml ultrapure water. The reaction mixture was then subjected to microwave irradiation in a domestic microwave oven (700 w) for about 4 minutes. When the mixture was cooled to room temperature, 25 mL of water was added to obtain a dark brown colored precipitate. This solution was centrifuged under 10000 rpm for 20 min. Then the supernatant was purified by dialysis (molecular weight cut off =1000).

Synthesis and modification Pt/CDs

200 μL aqueous solution of H_2PtCl_6 (1 wt %) was added to 1 mL aqueous suspension of CDs (1 mg/mL). The mixed solution was kept at 100 ^oC overnight to yield a black suspension of Pt/CDs. The suspension was centrifuged and washed three times with water. The obtained Pt/CDs was blocked by PEG-SH and then incubated in a 10 mM aqueous solution of $ZrOCl_2$ for 1 h, So that Zr^{4+} can bind to phosphonic acid groups of on the carbon dot. The final solution was again centrifuged and washed to remove any unbound Zr^{4+} and stored at 4 ^oC for further use.

Characterization of CDs and CD/PtNPs

The synthesized CDs exhibit characteristic absorbance and photoluminescence (PL) properties (Fig. S3). A sharp absorption band at 274 nm was observed in the UV-Vis spectrum (Fig. S3a), which is ascribed to the π–π* transition of the C=C structure. PL of CDs (Fig. S3b, c) shows the clear excitation wavelength dependence of the emission wavelength and intensity. Upon the optimal excitation at 420 nm, CDs emitted bright green fluorescence centered at 525 nm. Our FTIR results showed that phosphorus groups could be found in both CDs and Pt/CDs (Fig. S5). In FTIR (Fig. S5), the band located at 3400 cm^{-1} is ascribed to the hydroxyl (O-H) group. The broad peak between 2000 and 2500 cm^{-1} was typical for phosphate. Peaks at 1207 cm^{-1}, 1052 cm^{-1}, and 977 cm^{-1} were related to the vibration of P-O, P–O–R (R=alkyl) and P–O–H respectively.

Detection of PKC-α activity

Dephosphorylated MBP was first immobilized onto a polystyrene 96-well microplate in a 100

μL buffer with low ionic strength (0.2 mM Tris base, pH 7.4, 6 mM NaCl and 0.4 mM $MgCl_2$), overnight at 4 °C. The wells were washed three times with 100 μL buffer and then blocked with 300 μL, 1 % BSA for 30 min. The wells were then washed and incubated with 1 μM ATP, different units of PKC-α, 5 μL PKC lipid activator (1:20 reaction volume) for 1 h at 37 °C. Then 1% BSA blocked Pt/CD -Zr were applied at shaker for 30 min. Colorimetric measurements were conducted at plate reader with TMB and H2O2 reaction. Two control reactions were performed, one without PKC lipid activator and the other with different amount of inhibitor (GF109203X).

Detection of HER2 and phosphorylated HER2 (pHER2) on cell surface

30 μL cells were added in each well and cultured overnight at 37 °C in a 5 % CO_2 atmosphere. To detect pHER2, the cells were first treated with or without 100 ng·mL^{-1} EGF and 5 mM CI-1033 for 1 h and then fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100. After blocking with 2 % BSA, 50 μg mL^{-1} Pt/CDs were added in each well, incubated for 30 min and washed three times with PBS buffer. After that, Freshly prepared TMB and H_2O_2 was added for color development.

Immunohistochemistry staining

To stain cells using peroxidase-like activity, the wells seeded with cells were washed with PBS buffer, fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100. After blocking with 2 % BSA, 50 μg mL^{-1} Pt/CDs were added in each well, incubated for 1 h and washed three times with TBS buffer. Freshly prepared DAB and H_2O_2 was added for color development

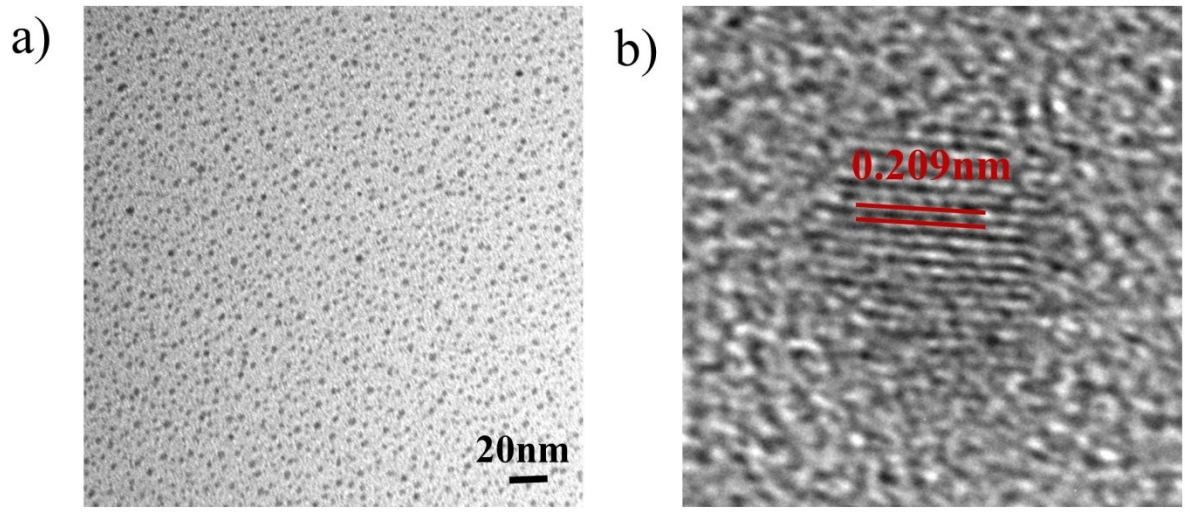

Figure S1. a) TEM and b) HRTEM images of as prepared CDs.

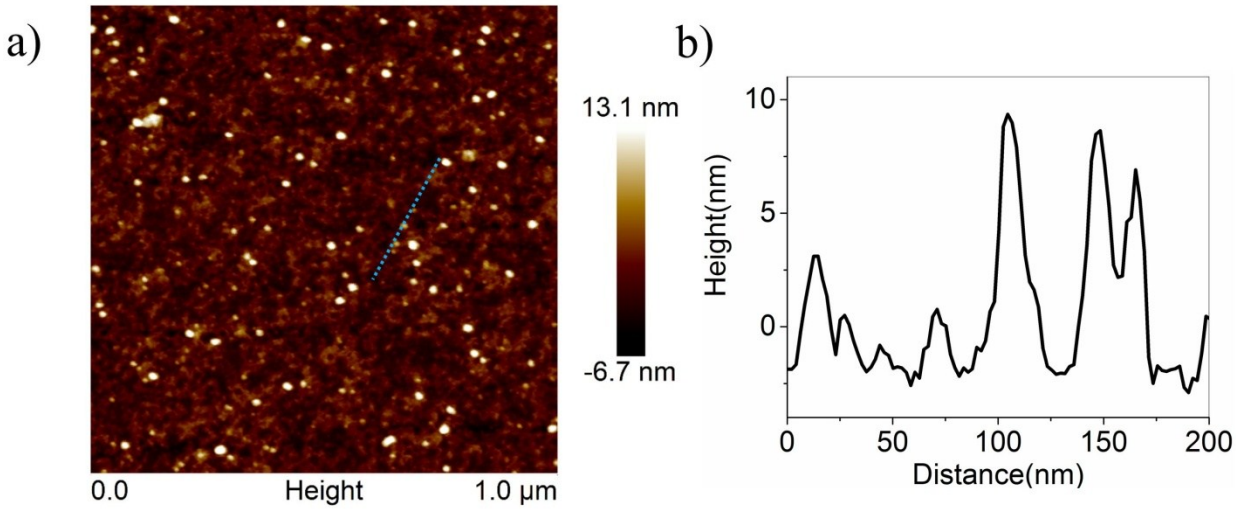

Figure S2. AFM image a) and height profile b) of CDs.

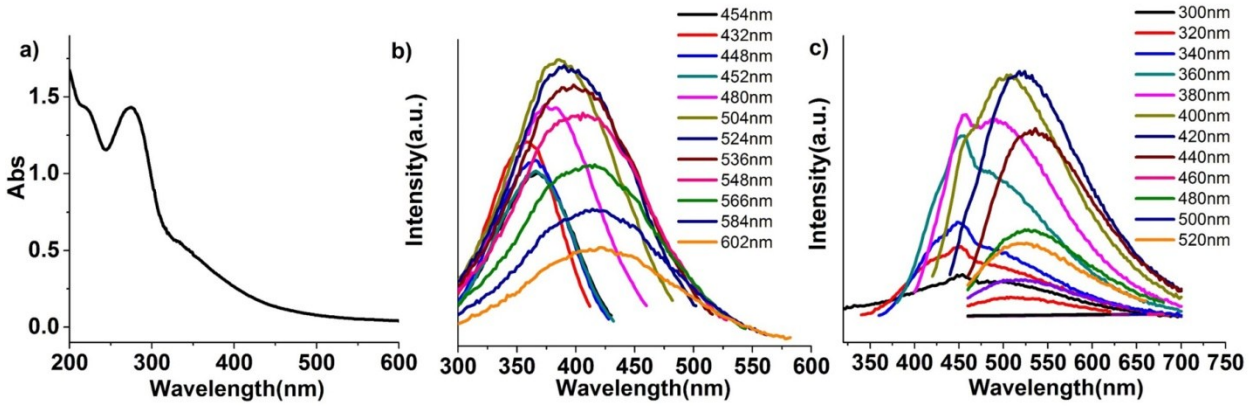

Figure S3. UV-Vis absorption a) and photoluminescence excitation b) and emission c) spectra of CDs.

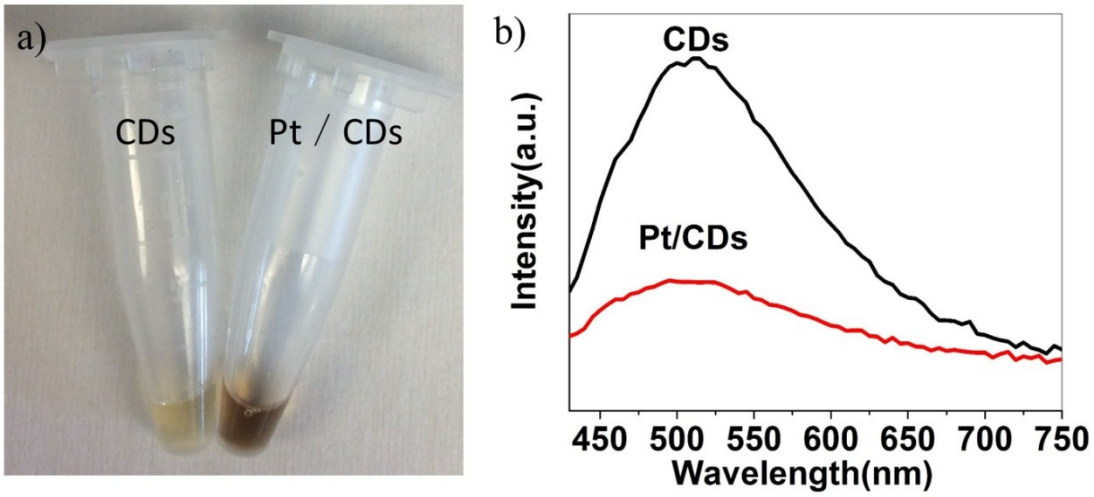

Figure S4. Digital image a) and photoluminescence emission spectra of CDs and Pt/CDs (b). The excitation wavelength is 420nm.

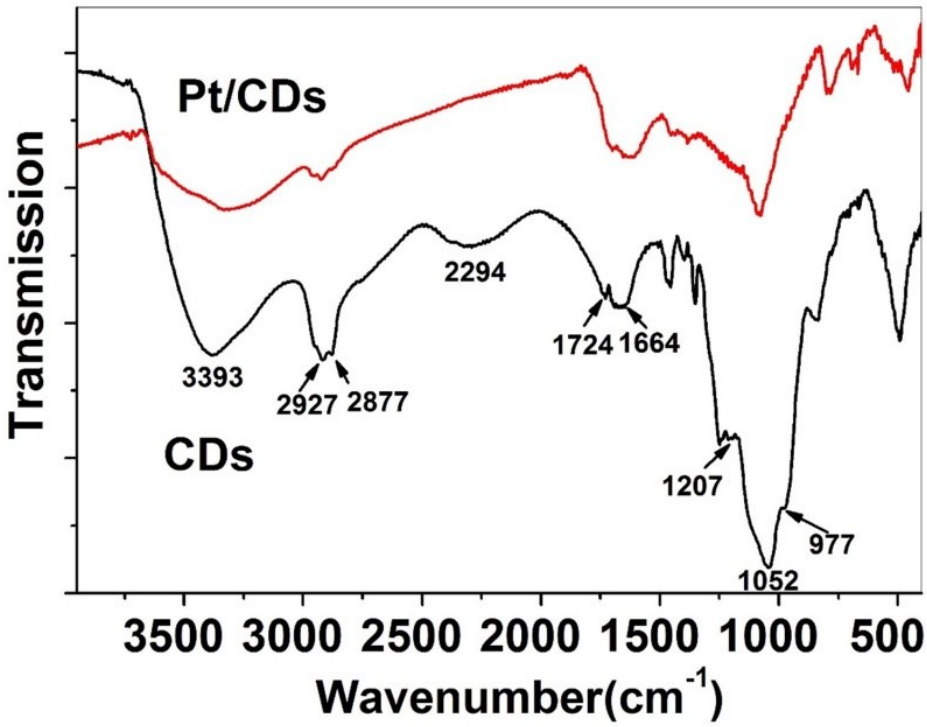

Figure S5. FTIR spectra of CDs and Pt/CDs.

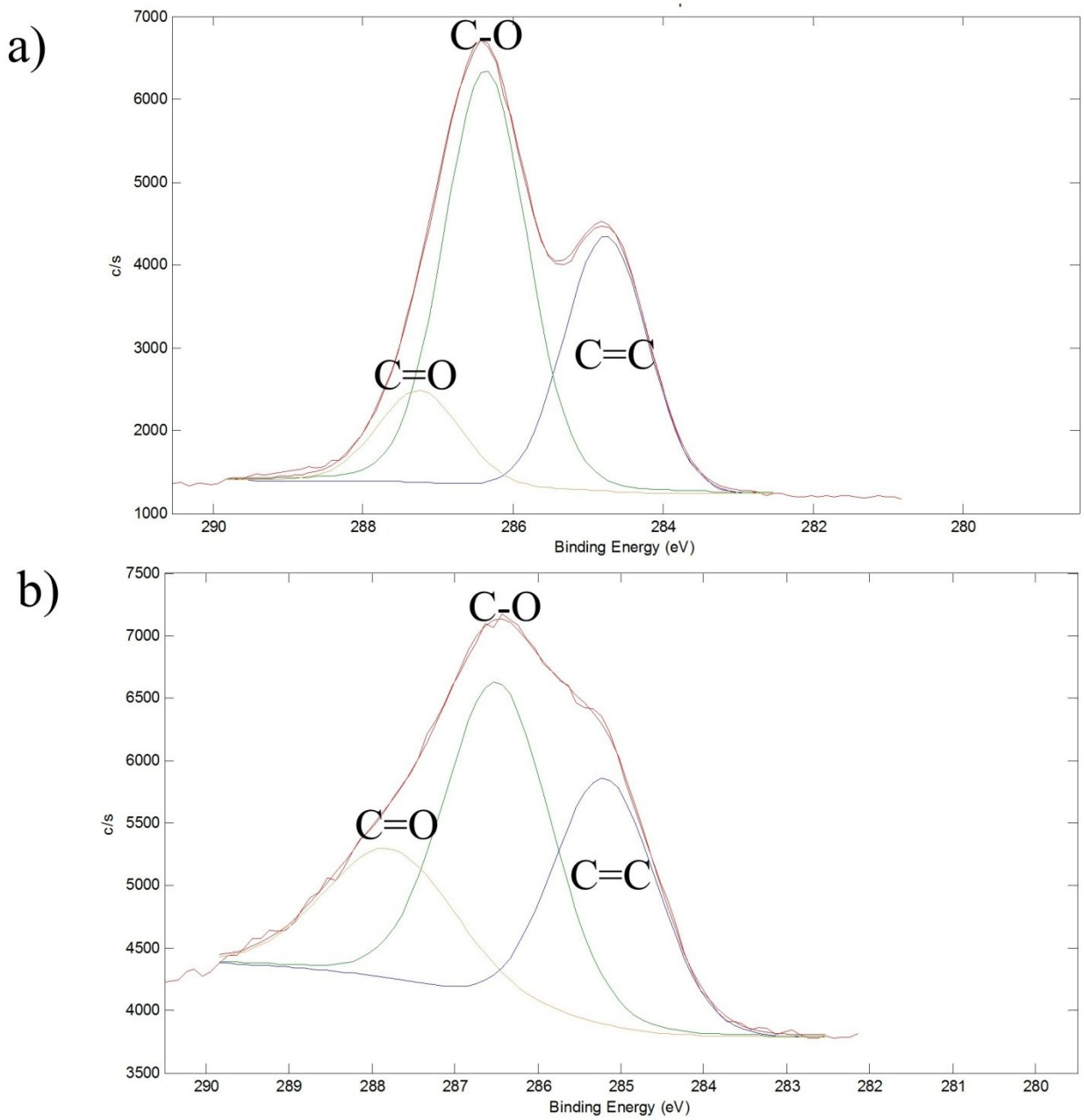

Figure S6. XPS C 1s spectra of the a) CDs and b) Pt/CDs.

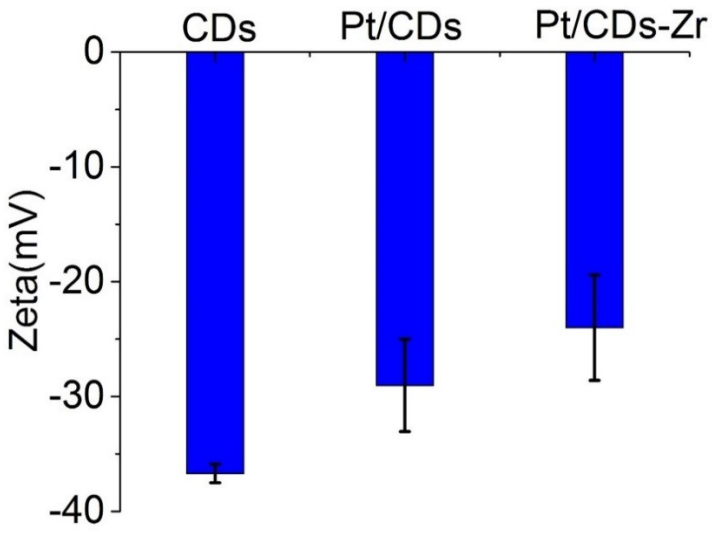

Figure S7. Zeta potential of CDs, Pt/CDs and Pt/CDs-Zr.

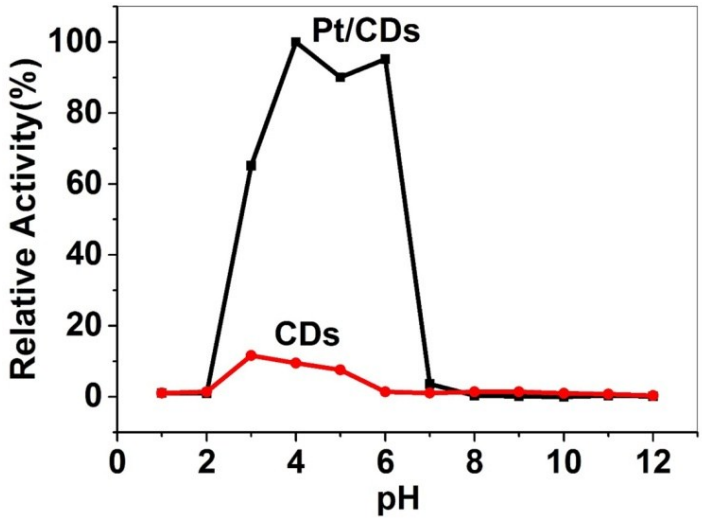

Figure S8. pH dependent of the peroxidase-like activity of CDs and Pt/CDs.

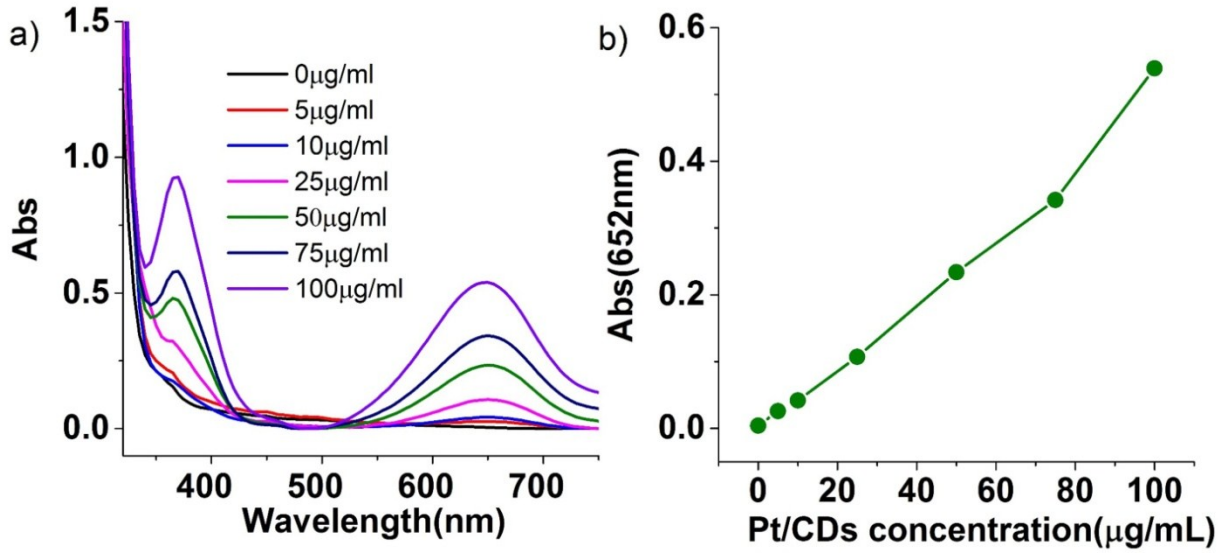

Figure S9. UV-vis spectra at 5 min a) and calibration curve at 652 nm b) show the concentration dependent of the peroxidase-like activity of Pt/CDs.

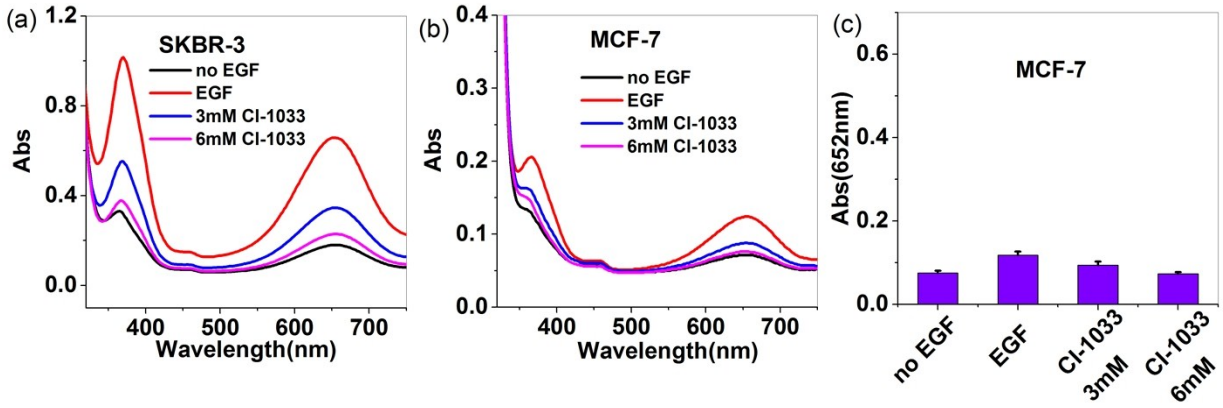

Figure S10. UV-Vis spectra at 5 min for Pt/CDs-based colorimetric detection of pHER2 expression on SKBR-3 a) and MCF-7 b) cells treated without or with 100 ng/mL EGF, 100 ng/mL EGF+3 mM CI-1033 and 100 ng/mL EGF+6 mM CI-1033. c) Detection of phosphorylated HER2 (pHER2) expression on SKBR-3 and MCF-7 cells treated without or with 100 ng/mL EGF, 100 ng/mL EGF+3 mM CI-1033 and 100 ng/mL EGF+6 mM CI-1033.