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# Supplementary Information

## A label-free and blocker-free photoelectrochemical strategy for highly sensitive caspase-3 assay

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## 1. Materials and reagents

Human recombinant caspase-3 was purchased from R&D Systems (Minneapolis, USA). Caspase-3 Inhibitor (Ac-DEVD-CHO) was obtained from Beyotime Institute of Biotechnology (Haimen, China). The CC-DEVD-peptide (Ac-Gly-Asp-Gly-Asp-Glu-Val-Asp-Cys-Cys-$NH_2$) and the C-DEVD-peptide (Ac-Gly-Gly-Asp-Gly-Asp-Glu-Val-Asp-Cys-$NH_2$) were obtained from GL Biochem. Ltd. (Shanghai, China). The ITO slices were purchased from Zhuhai Kaivo Electronic Components Co., Ltd, China. Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), bovine serum albumin (BSA) and dithiothreitol (DTT) were purchased from Sigma-Aldrich (USA). Tris-(hydroxymethyl) aminomethane (Tris), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), 2-methylimidazole, cadmium nitrate ($Cd(NO_3)_2 \cdot 4H_2O$), sodium sulfide ($Na_2S \cdot 9H_2O$), methanol and ascorbic acid (AA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Other chemicals were of analytical grade and used as received. All solutions were prepared with ultrapure $H_2O$ from a Milli-Q filtration system (Millipore Corp., Bedford, MA).

## 2. Apparatus

The morphology and structure of the prepared materials were investigated by scanning electron microscopy (SEM, JSM-6700F, Japan) and transmission electron microscopy (TEM, Tecnai G2 F20 S-TWIN, Holland). The UV-vis spectra were recorded by a UV-2500 UV-vis spectrophotometer (LabTech, China). Powder X-ray diffraction (PXRD) was recorded on an X-ray diffractometer (PXRD, D/MAX-RA,

Japan). Nitrogen adsorption–desorption isotherms and Brunauer–Emmett–Teller (BET) surface area of the samples were measured by a Micrometrics sorptometer (ASAP 2020, USA). The zeta potentials of the samples were investigated on a Zetasizer Nano ZS potential analyzer. A PLS-SXE300 Xe lamp equipped with an optical filter ($\lambda > 420$ nm) was used as the light source. PEC and electrochemical measurements were carried out on a CHI 660D electrochemical workstation at room temperature. A typical three-electrode system consisted of a modified ITO electrode (5.6 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. All potentials in this paper were referred to SCE.

## 3. **Preparation of the PEC sensor**

The NPC-ZnO nanopolyhedra were prepared according to our previous paper.[1] Prior to electrode modification, ITO electrode was cleaned by ultrasonic treatment in acetone, 1 M NaOH in ethanol/water mixture (v/v, 1:1) and water for 15 min each. After dried at 60 °C, 25 μL of NPC-ZnO nanopolyhedra suspension (1 mg/mL) was dropped onto the surface of the cleaned ITO electrode and dried in air. The deposition of CdS on the ITO/NPC-ZnO electrode was carried out by the successive ionic layer adsorption and reaction (SILAR) method with some modifications.[2] In brief, the ITO/NPC-ZnO electrode was dipped into 0.1 M $Na_2S$ methanol/water mixture (1:1, v/v) for 1 min and washed with methanol, then followed by dipping into 0.1 M $Cd(NO_3)_2$ methanol solution for 1 min, and again rinsed with methanol. After two

cycles of this dipping process, the ITO/NPC-ZnO/CdS electrode was obtained.

Before immobilized onto the ITO/NPC-ZnO/CdS electrode surface, the CC-DEVD-peptide was dissolved in 0.1 M acetate buffer solution (containing 10 mM TCEP, pH 5.2) and incubated for 1 h to prevent terminal cysteine from the formation of disulfide bonds. Then, 20 μL of CC-DEVD-peptide solution (1 mM) was placed onto the ITO/NPC-ZnO/CdS electrode for 12 h at 4 °C to obtaine the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode. For comparison, ITO/NPC-ZnO/CdS/C-DEVD-peptide electrode was also fabricated with the same procedure.

## 4. **PEC Assay of Caspase-3**

Assay buffer solutions (20 μL, pH 7.4, 25 mM HEPES, 0.1% CHAPS and 10 mM DTT) containing different concentrations of caspase-3 were dropped onto the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrodes, and then incubated for 20 min at 37 °C. The obtained electrodes were rinsed with Tris-HCl buffer (0.1 M, pH 7.4) and then PEC measurements were carried out in Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 M AA at −0.3 V.

## 5. **Characterization of NPC-ZnO/CdS Hybrids**

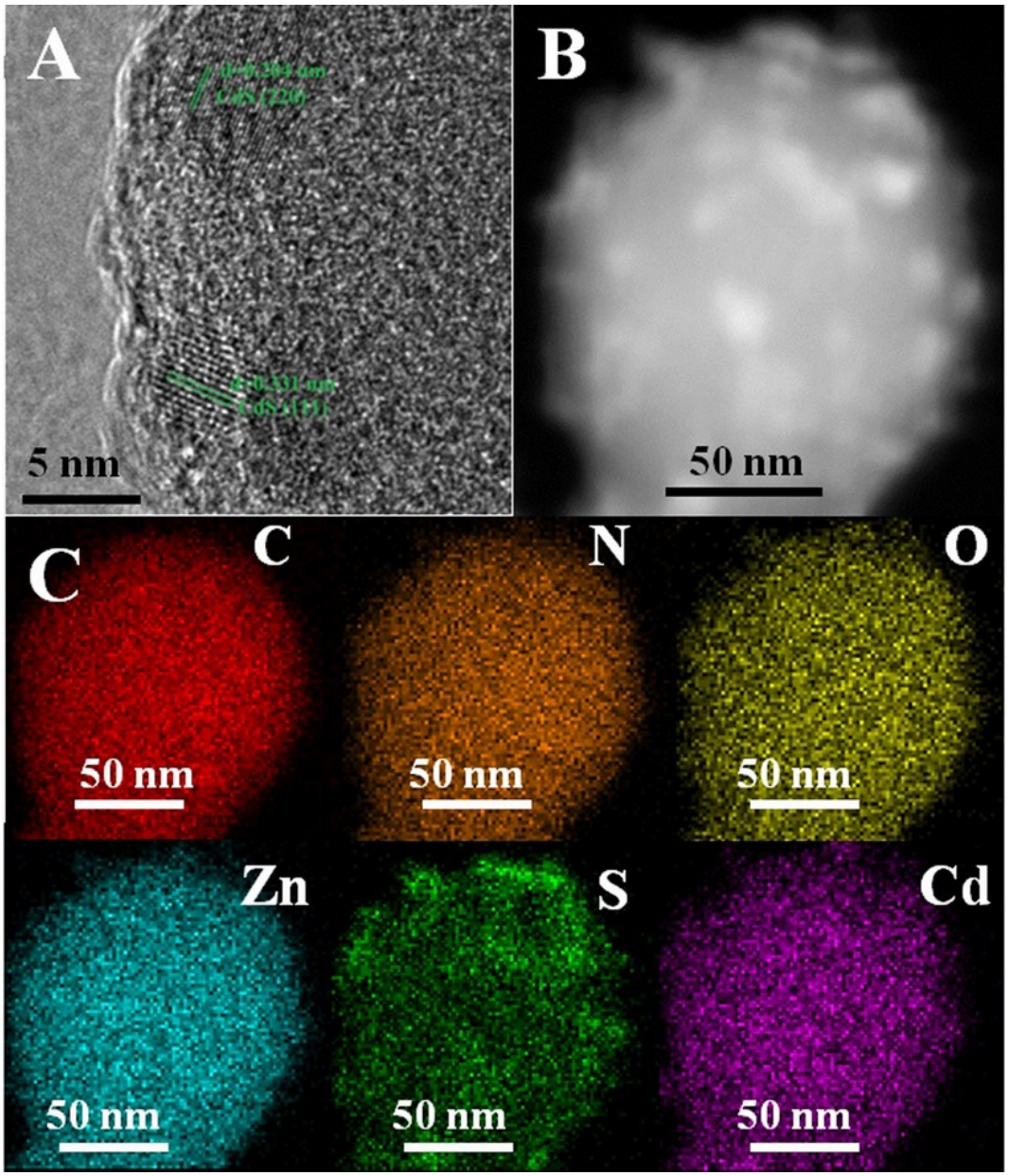


**Fig. S1.** HRTEM images of the proposed NPC-ZnO/CdS hybrids (A). EDS mappings in TEM for the NPC-ZnO/CdS hybrids (B) with C, N, O, Zn, S, and Cd (C).

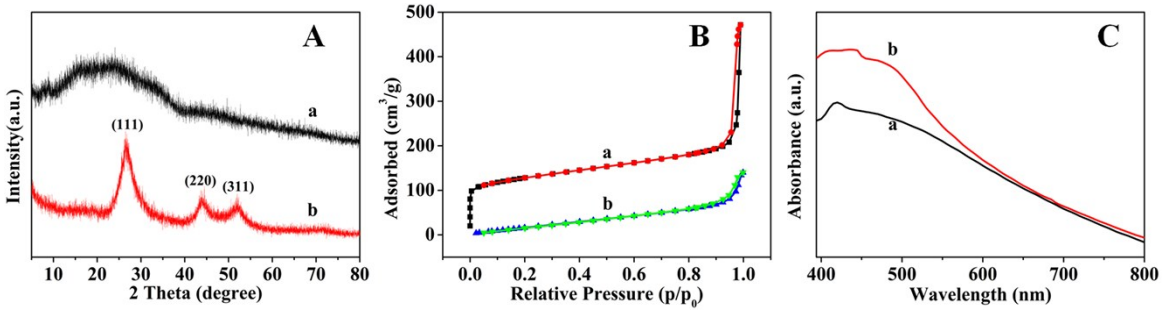


**Fig. S2**. (A) PXRD patterns, (B) Nitrogen adsorption-desorption isotherms, and (C) UV-vis absorption spectra of the NPC-ZnO nanopolyhedra (a) and NPC-ZnO/CdS hybrids (b).

## 6. EIS and PEC Characterization of the Caspase-3 Sensor

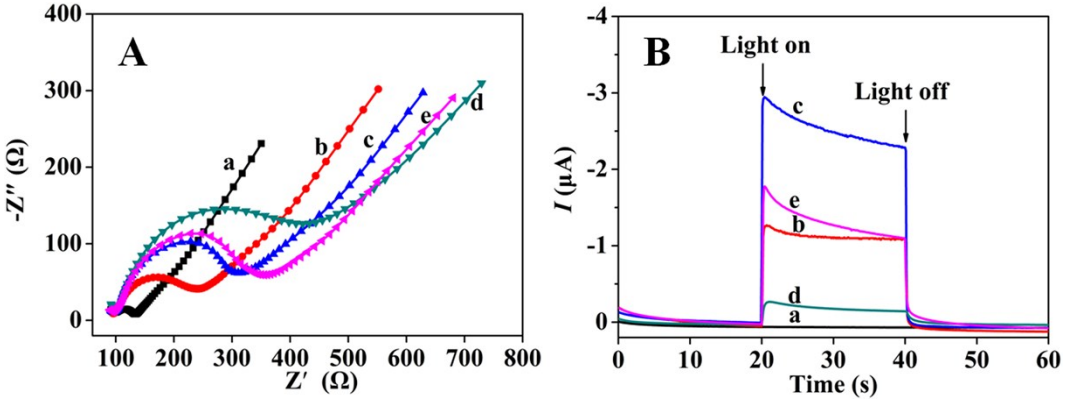


**Fig. S3**. (A) EIS results of the different modified ITO electrodes in 0.1 M KCl aqueous solutions containing 5 mM (1:1) $[Fe(CN)_6]^{3-/4-}$ at -0.22 V in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude. (B) Photocurrents of the different modified ITO electrodes in 0.1 M Tril-HCl (pH 7.4) containing 0.1 M AA. (a) ITO electrode, (b) ITO/NPC-ZnO electrode, (c) ITO/NPC-ZnO/CdS electrode, (d) ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode, (e) ITO/NPC-ZnO/CdS/ CC-DEVD-peptide electrode after incubation with 20 μL caspase-3 (50 ng/mL).

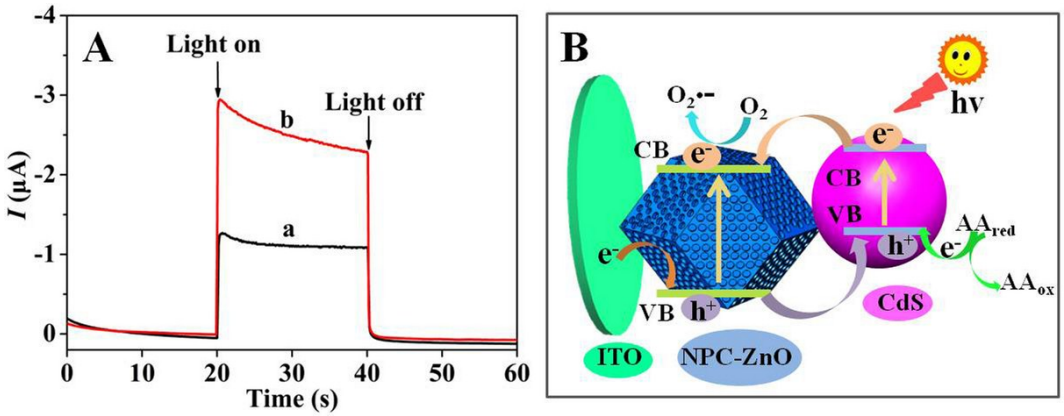


**Fig. S4.** (A) Photocurrents of (a) ITO/NPC-ZnO and (b) ITO/NPC-ZnO/CdS electrodes. (B) The possible photocurrent generation mechanism of ITO/NPC-ZnO/CdS electrode.

A possible photocurrent generation mechanism of the NPC-ZnO/CdS hybrids is illustrated in Fig. S4B. Under visible light illumination, both the NPC-ZnO nanopolyhedra and CdS are easily excited and correspondingly photo-generated electrons/holes are formed on the conduction band (CB)/valence band (VB), respectively. The photo-generated electrons on the CB of CdS are moved to the CB of the NPC-ZnO nanopolyhedra for the electrochemical reduction of $O_2$ (electron acceptors) in the electrolyte. Meanwhile, AA can effectively be oxidized by photo-generated holes to reduce the recombination of photo-generated electron-hole pairs and to promote the generation of photo-generated electrons, resulting in the enhanced photocurrent.[1,3]

## 7. Optimization of experimental conditions

To achieve the good analytical performance of the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode for caspase-3 assay, several experimental parameters, including the NPC-ZnO concentration, the cycle number of the CdS SILAR, the CC-DEVD-peptide concentration and the reaction time between caspase-3 and CC-DEVD-peptide, were optimized.

The effect of the concentration of the NPC-ZnO nanopolyhedra on the photocurrent of the ITO/NPC-ZnO electrode was investigated and the corresponding results are shown in Fig. S5A. It is noted that the photocurrent of the ITO/NPC-ZnO electrode increases with the increase of the concentration of NPC-ZnO nanopolyhedra and a platform reaches at 1.0 mg/mL. Thus, 1.0 mg/mL is selected as the optimal concentration of the NPC-ZnO nanopolyhedra.

The amount of CdS on the NPC-ZnO nanopolyhedra is one of the key factors on the PEC properties of the NPC-ZnO/CdS hybrids. Thus, the SILAR cycle number has been investigated. As shown in Fig. S5B, the photocurrent of ITO/NPC-ZnO/CdS electrode increases with the increase of the SILAR cycle number and a maximum is observed at 2. When the SILAR cycle number is more than 2, the photocurrent of ITO/NPC-ZnO/CdS electrode decreases with the increase of the SILAR cycle number. The possible reason is as follows: the excessive CdS particles offer more surface recombination centers which obviously increase the diffusion resistance for electron motion, and therefore the photocurrent decreases.[4] Thus, two SILAR cycles of CdS is adopted to fabricate the ITO/NPC-ZnO/CdS electrode.

The concentration of the CC-DEVD-peptide is another key factor for caspase-3 assay. From Fig. S5C, it is clear that the photocurrent of the electrode decreases with the increase of CC-DEVD-peptide concentration and then reaches a plateau at 1 mM. So, 1 mM is chosen as the optimal concentration of CC-DEVD-peptide.

The reaction time between caspase-3 and CC-DEVD-peptide is also optimized. As shown in Fig. S5D, when the reaction time between caspase-3 and CC-DEVD-peptide increases, more CC-DEVD-peptide can be cleaved, leading to the increased photocurrent. And a plateau reaches at 20 min. Thus, 20 min is taken as the optimal reaction time between caspase-3 and CC-DEVD-peptide.

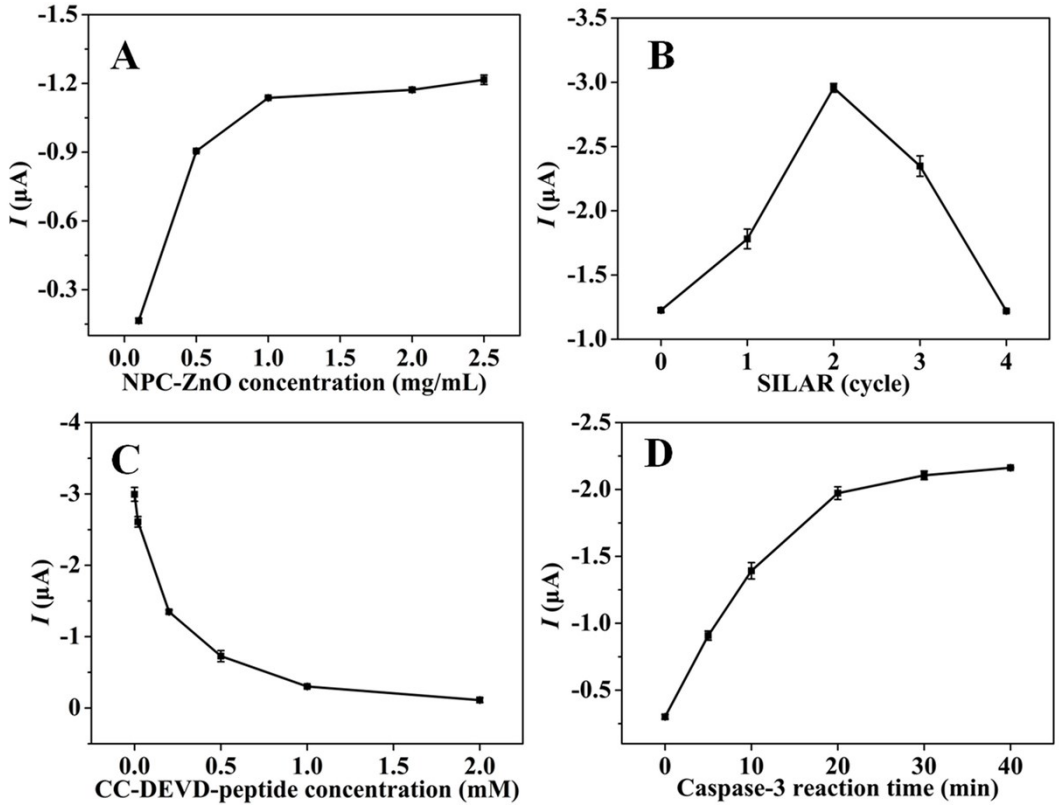


**Fig. S5.** The effects of different parameters on the photocurrent responses of the modified electrodes. (A) The effect of NPC-ZnO concentration on the photocurrent of the ITO/NPC-ZnO electrode; (B) the effect of CdS SILAR cycle number on the

photocurrent of the ITO/NPC-ZnO/CdS electrode (NPC-ZnO concentration, 1 mg/mL); (C) the effect of CC-DEVD-peptide concentration on the photocurrent of the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode (NPC-ZnO concentration, 1 mg/mL; CdS SILAR cycle number, 2 cycles); (D) the effect of the reaction time between caspase-3 and CC-DEVD-peptide on the photocurrent of the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode after incubated with caspase-3 (NPC-ZnO concentration, 1 mg/mL; CdS SILAR cycle number, 2 cycles; CC-DEVD-peptide concentration, 1 mM; caspase-3 concentration, 200 ng/mL).

## 8. **Selectivity, Reproducibility and Stability**

In order to investigate the selectivity of the proposed method, the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrodes were incubated with interfering proteins, including alkaline phosphatase (ALP), pepsin, glucose oxidase ($GO_X$), lysozyme and BSA, respectively. The concentrations of the interfering proteins (10000 ng/mL) are much higher than that of caspase-3 (50 ng/mL). From Fig. S6A, it is noted that no significant photocurrent responses are observed when the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode are incubated with the interfering protein solutions. However, a large photocurrent response can be observed when the electrode is incubated with caspase-3. This can be ascribed to the fact that caspase-3 can specifically recognize and cleave CC-DVED-peptide. In order to make sure the selectivity of the PEC sensor, an inhibitor of caspase-3, Ac-DEVD-CHO, was used.

Caspase-3 is pre-incubated with 100 μM Ac-DEVD-CHO for 30 min before reacting with CC-DEVD-peptide. As shown in Fig. S6B, a small photocurrent (column b) is observed, indicating good inhibiting effect of Ac-DEVD-CHO on the caspase-3 activity. This also implies that the developed ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode has high selectivity toward the caspase-3 assay and can also be used to quickly screen the related inhibitors of caspase-3.

The reproducibility of the developed PEC sensor is also evaluated. For 50 ng/mL caspase-3, the relative standard deviation (RSD) of 4.3% is obtained for five ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrodes. This demonstrates the satisfactory reproducibility of the proposed method. Additionally, the stability of the ITO/NPC-ZnO/CdS/CC-DEVD-peptide electrode is further investigated. The photocurrent response of the ITO/NPC-ZnO/CdS/DEVD-peptide electrode could retain about 94.1% of its initial photocurrent response toward caspase-3 assay after 15 days of storage in a refrigerator at 4 °C, indicating an acceptable stability of the electrode.

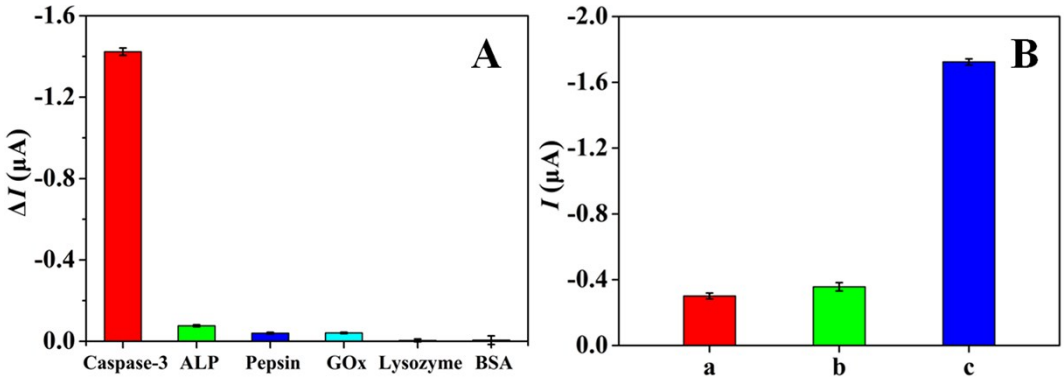


**Fig. S6**. (A) Selectivity of the PEC sensor for caspase-3 assay. Caspase-3 concentration, 50 ng/mL; the concentration of other proteins (ALP, pepsin, $GO_X$, lysozyme and BSA), 10000 ng/mL. $\Delta I = I - I_0$, where $I$ and $I_0$ represent the

photocurrents of the electrode in the presence and absence of different protein (caspase-3, or ALP, pepsin, $GO_X$, lysozyme, BSA), respectively. (B) PEC assay for caspase-3 inhibition by Ac-DEVD-CHO based on the ITO/NPC-ZnO/CdS/DEVD-peptide electrode. (a) without caspase-3 and Ac-DEVD-CHO, (b) 50 ng/mL caspase-3 and 100 μM Ac-DEVD-CHO, (c) 50 ng/mL caspase-3.

## 9. Recovery test

To further evaluate the practical application in complex biological system, the developed PEC sensor was carried out to analysis caspase-3 in cell extracts. It is noted that no obvious photocurrent response of the electrode can be observed for 100-fold diluted Human breast adenocarcinoma (MDA-MB-231) cell extracts (friendly provided by Nie's group in Hunan University and used as received without any pretreatment, diluted by 25 mM HEPES, 0.1% CHAPS and 10 mM DTT (pH 7.4)). After that, caspase-3 with different concentrations (1, 10 and 20 ng/mL) was spiked into the 100-fold diluted MDA-MB-231 cell extracts and detected by the developed electrode. Here, the $\Delta I_r$ value ($\Delta I_r = I_r - I_{r0}$, where $I_r$ and $I_{r0}$ represent the photocurrents of the electrode for 100-fold diluted MDA-MB-231 cell extracts spiked with and without caspase-3, respectively) was used to calculate the found concentration of caspase-3 spiked in cell extracts. As shown in Table S1, the recoveries in cell extracts for the added caspase-3 with 1, 10 and 20 ng/mL are 102, 101 and 98%, respectively. These results clearly reveal that the recovery of the proposed CC-DEVD-peptide-based PEC sensor is satisfactory and has great potential

applications in caspase-3 assay.

**Table S1. Recovery assay of Caspase-3 in the Cell Extract Sample.**

| Sample | added (ng/mL) | found (ng/mL) | R.S.D. (%) | Recovery (%) |
|---|---|---|---|---|
| 1 | 1 | 1.02 | 1.57 | 102 |
| 2 | 10 | 10.08 | 5.50 | 101 |
| 3 | 20 | 19.62 | 5.02 | 98 |

## References


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