CdS:Mn quantum dot-functionalized g-C₃N₄ nanohybrids as signal-generation tags for photoelectrochemical immunoassay of prostate specific antigen coupling DNAzyme concatamer with enzymatic biocatalytic precipitation

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ABSTRACT

A new photoelectrochemical (PEC) immunosensor based on Mn-doped CdS quantum dots (CdS:Mn QDs) on g-C₃N₄ nanosheets was developed for the sensitive detection of prostate specific antibody (PSA) in biological fluids. The signal derived from the as-synthesized Cd:Mn QDs-functionalized g-C₃N₄ nanohybrids *via* a hydrothermal method and was amplified through DNAzyme concatamers on gold nanoparticles accompanying enzymatic biocatalytic precipitation. Experimental results by UV-vis absorption spectra and photoluminescence revealed that CdS:Mn QDs/g-C₃N₄ nanohybrids exhibited higher photocurrent than those of CdS:Mn QDs and g-C₃N₄ alone. Upon addition of target PSA, a sandwich-type immunoreaction was carried out between capture antibodies and the labeled detection antibodies. Accompanying introduction of gold nanoparticles, the labeled initiator strands on the AuNPs triggered hybridization chain reaction and the formation of DNAzyme concatamers in the presence of hemin. The formed DNAzyme catalyzed 4-chloro-1-naphthol (4-CN) to produce an insoluble/insulating precipitate on the Mn:CdS QDs/g-C₃N₄, and blocked the light harvesting of Mn:CdS QDs/g-C₃N₄, thus resulting in the decreasing photocurrent. Under optimal conditions, the immunosensor exhibited good photocurrent responses for determination of target PSA, and allowed detection of PSA at a concentration as low as 3.8 pg mL⁻¹. The specificity, reproducibility and precision of this system were acceptable. Significantly, this methodology was further evaluated for analyzing human serum samples, giving well-matched results with referenced PSA enzyme-linked immunosorbent assay (ELISA) method.

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KEYWORDS: photoelectrochemical immunosensor; Mn-doped CdS quantum dots; g-C₃N₄ nanosheets; enzyme biocatalytic precipitation; DNAzyme concatamers; hybridization chain reaction

1. Introduction

Sensitive and accurate monitoring of low-abundance proteins in biological fluids played a very important role in the early disease diagnosis (Yin et al., 2010). The abnormal levels of proteins (*e.g.*, tumor markers released from healthy cells and overexpressed in tumor tissue) are closely associated with the stages of cancers (Kerman et al., 2007). As a diffusely used tumor marker, prostate specific antigen (PSA) has been known to be the most specific and efficient biomarker for the screening and diagnosis of prostate disorders (*e.g.*, prostate cancer), and the normal levels of PSA are lower than 4 ng mL⁻¹ in serum (Wu et al., 2007). The increased PSA concentration is commonly suspected to be connected with prostate cancer or other prostate diseases. Recently, different methods have been reported for PSA detection, *e.g.*, (electro-)chemiluminescence immunoassay, enzyme immunoassay, fluorescence immunoassay, electrochemical immunoassay and radioimmunoassay (RIA) (Xu et al., 2017; Shi et al., 2016; Acevedo et al., 2002; Shu et al., 2016; Zhuang et al., 2015; Graves et al., 1992). Despite the high sensitivity of conventional immunoassays such as RIA and ELISA, they have some limitations, such as the short shelf life of ¹²⁵I-labeled antibody, radiation hazards, low sensitivity and expensive instruments. Hence, it is necessary to develop sensitive analytical methods to meet the needs for detection of low-concentration biomarkers and remedy of cancer, especially in the early cancer stage because the number of tumor cells is too small to produce a large number of biomarkers (Wang et al., 2015; Ge et al., 2016).

Photoelectrochemical (PEC) analysis, an emerging and rapidly developing detection technique for the biological assay with the advantages of simple operation, inexpensive equipment and good portability in recent years, has attracted worldwide attention. Owing to the different energy forms of the excitation source/light and the readout signal/electricity, PEC method shows lower background signal and higher sensitivity than traditional electrochemical methods (Li et al., 2012; Zhao et al., 2014). Nowadays, the choice of photoactive materials (*e.g.*, SnO₂, TiO₂, CdS and CdTe) plays an important role in the design of PEC sensors. The properties of the photoactive semiconductors have a strong impact on the analysis performance of the sensors. Graphitic carbon nitride (g-C₃N₄), a metal-free and nontoxic semiconductor with high thermal and chemical stability, has attracted a

great deal of research interest due to its excellent photocatalytic activity (Wang et al., 2012; Li et al., 2014; Wang et al., 2016), and has been extensively studied in the fields of catalysis (Wang et al., 2009; Yang et al., 2011), sensors (Lee et al., 2010; Zhang et al., 2014) and energy storage (Tahir et al., 2013). Unfortunately, the practical application of pure g-C₃N₄ was limited to some extent because of its low photon-to-electron conversion efficiency that caused by the high recombination rate of electron-hole pairs (Zhang et al., 2010). Accordingly, many innovative methods, *e.g.*, by doping with metal or nonmetal elements (Yan et al., 2010; Lu et al., 2014), have been developed to improve the conversion efficiency of g-C₃N₄. CdS quantum dot (QD), an eminent semiconductor material with a narrow band gap (~2.4 eV), has been intensively investigated owing to its excellent PEC activity (Zhou et al., 2014; Song et al., 2016). However, pure CdS QD, the same as g-C₃N₄, also frequently suffers from the high recombination rate of electron-hole pairs (Shen et al., 2015). Due to the stable nanostructure and large surface area of g-C₃N₄ and the well-matched overlapping band-structures between g-C₃N₄ and CdS QD (Fu et al. 2013), it would be advantageous for the construction of heterojunction structures coupling with g-C₃N₄ and CdS QD because it effectively suppresses the recombination of the electron-hole pairs on the semiconductors and extends their light-harvesting ability. To this end, our motivation of this work is to fabricate the nanocomposites consisting of g-C₃N₄ and CdS:Mn QDs. The doped Mn²⁺ ions can produce an electronic state in the mid-gap region of the CdS crystal (Wu et al., 2015) to promote the separation of the photogenerated electron-hole pairs, thereby increasing the photocurrent.

Signal amplification is another important factor in obtaining a high-sensitivity PEC sensor. To date, many immunoassays based on the signal amplification with the use of natural enzymes have been developed (Zhao et al., 2012; Sun et al., 2014; Shu et al., 2015). Due to the inherent properties of natural enzymes, they are sensitive to environmental conditions and are prone to denaturation and inactivation. Thus, the application of natural enzymes in PEC analysis is limited. In contrast, DNAzymes, which are DNA molecules with a catalytic activity, could replace the natural enzyme to catalyze the same reaction. Furthermore, the synthesis of DNAzymes is very easy and inexpensive besides the good stability. Hemin/G-quadruplex, one of DNAzymes that has peroxidase-like activity, has been widely used in sensors (Xu et al., 2015; Du et al., 2016; Qiu et al., 2017). For example, our group reported an electrochemical immunosensor based on hemin/G-quadruplex-based DNAzyme concatamers for the signal amplification (Tang et al., 2012). DNA concatamer, a linear long-chain

formed by self-assembly of short DNA fragments, has many dendritic single-stranded DNAs on its long-chain structures. Dendritic single-stranded DNA can be designed to be rich in G bases so that hemin can be bound to the DNA strand to form hemin/G-quadruplex. In this case, the formed DNA concatamer contains a lot of DNAzymes for the signal amplification.

Herein, we report the proof-of-concept of a new “signal-off” PEC biosensor for detection of PSA based on DNAzyme concatamers and hemin/G-quadruplex-assisted biocatalytic precipitation, using CdS:Mn/g-C₃N₄ nanohybrids as photoactive materials (Scheme 1). The immunoreaction is carried out on capture antibody (Ab₁)-immobilized electrode (modified with CdS:Mn/g-C₃N₄ nanohybrids), using the initiator strand and detection antibody-conjugated gold nanoparticle (S₀-AuNP-Ab₂), with a sandwich-type immunoassay format. Thereafter, the carried initiator strand accompanying the immunocomplex triggers the hybridization chain reaction between single-strand DNA₁ (S₁) and single-strand DNA₂ (S₂) to form DNA concatamer. Upon addition of hemin, DNAzyme is formed on the concatamer to execute enzymatic biocatalytic precipitation toward 4-chloro-1-naphthol, thus resulting in the decreasing photocurrent of the modified electrode. By monitoring the change of the photocurrent, we can quantitatively determine the concentration of target PSA in the sample.

Scheme 1. (A) Mechanism of the photocurrent generation of g-C₃N₄/CdS:Mn under visible-light irradiation, and (B) schematic illustration of photoelectrochemical immunosensing system.

2. Experimental

Scheme 1 gives the schematic illustration of the photoelectrochemical immunosensor toward target PSA. Partial experimental procedures were described in detail in the Supporting Information.

Electrochemical measurement was performed on a homemade equipment (with a 500 W Xe lamp as the irradiation source) and a CHI 630D electrochemical workstation (CH Instruments Inc., Shanghai, China) in 0.1 M pH 7.4 PBS containing 0.1 M KCl, including a conventional three-electrode system with an $\text{Ab}_1/\text{g-C}_3\text{N}_4/\text{CdS:Mn}$ -modified glassy carbon electrode (GCE) as the working electrode, a Pt-wire auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. The experimental procedures of PEC assay were performed as follows: (i) 10 μL of incubation solution including various-concentration PSA samples and $\text{S}_0\text{-AuNP-Ab}_2$ was added onto the surface of the modified electrode and incubated for 40 min at room temperature; (ii) 20 μL of DNA hybridization solution containing 0.4- μM S_1 and 0.4- μM S_2 was dropped onto the electrode, and reacted for 100 min; (iii) the abovementioned electrode was immersed into 0.2 mM hemin solution and incubated for 30 min; (iv) the electrode was dipped into 0.1 M PBS (pH 7.4) containing 1.0 mM 4-CN and 0.15 mM H_2O_2 for 40 min; and (v) the resulting electrode was introduced to photocurrent measurement referring to aforementioned assay system.

3. Results and discussion

3.1. Morphology and spectroscopic characterizations

The morphology and surface structure of $\text{g-C}_3\text{N}_4$, CdS:Mn QDs and $\text{g-C}_3\text{N}_4/\text{CdS:Mn}$ composites were observed by transmission electron microscopy (TEM). As seen from Fig. 1A, pure $\text{g-C}_3\text{N}_4$ sample displayed a typical planar sheet-like morphology with an irregular shape, consistent with the previous literatures (Fu et al. 2013; Niu et al. 2012), demonstrating the successful synthesis of $\text{g-C}_3\text{N}_4$. CdS:Mn QDs exhibited a granular structure with an average size of 6 nm (Fig. 1B), which was different from $\text{g-C}_3\text{N}_4$. The inset of Fig. 1B shows the high-resolution TEM (HRTEM) image of the CdS:Mn QDs sample, the lattice fringes are clearly exhibited with an interplanar distance of 0.341 nm, which could be attributed to the (111) crystal planes of CdS. As shown in Fig. 1C, the energy dispersive X-ray spectrum of CdS:Mn QDs distinctly verify the existence of Mn, Cd and S elements. The strong peaks for Cd and S elements were found in the spectrum, due to its abundant amount in the composites. Moreover, the presence of Mn in the spectrum (two peaks) revealed that the doping was successfully. It is well-known that the large superficial area of $\text{g-C}_3\text{N}_4$, which structure was derived from the graphite sheet, can afford effective loading sites for other

photoactive materials. As indicated from Fig. 1D, we observed that a large amount of CdS:Mn QDs were densely dispersed onto the surfaces of g-C₃N₄ nanosheet. In addition, HRTEM was employed to study the composites (Fig. 1E). A very close and distinguishable interface between g-C₃N₄ and CdS:Mn QDs in the composites was observed, which could facilitate the electron transfer and the separation of electron-hole pairs, thus promoted photoelectrical conversion efficiency.

Fig. 1. TEM images of (A) g-C₃N₄, (B) CdS:Mn QDs and (D) g-C₃N₄/CdS:Mn [note: HRTEM image of (B, inset) CdS:Mn QDs and (E) g-C₃N₄/CdS:Mn]; (C) EDX spectra of CdS:Mn QDs; (F) UV-vis absorption spectra of (a) g-C₃N₄, (b) g-C₃N₄/CdS:Mn, (c) CdS:Mn QDs and (d) CdS QDs; and PL spectra of (a') g-C₃N₄ and (b') g-C₃N₄/CdS:Mn [Inset: photographs of (left) g-C₃N₄ and (right) g-C₃N₄/CdS:Mn under excitation at 365 nm].

The optical properties of the as-prepared photoactive samples were measured by UV-vis absorption spectroscopy and photoluminescence (PL) spectroscopy. Fig. 1F (blue lines) compares the absorbance properties of the pure g-C₃N₄, CdS QDs, CdS:Mn QDs and g-C₃N₄/CdS:Mn. Pure g-C₃N₄ showed a strong absorption peak at 310 nm with a range from 300 to 400 nm (curve a), indicating a good light-harvesting. Compared with CdS:Mn QDs and CdS QDs (curves c-d), the absorbance of CdS QDs was much lower than that of CdS:Mn QDs, it could be determined that the formed CdS:Mn doping structure could enhance the absorption of light. Owing to the formation of

nanocomposites, curve *b* exhibited a broad and strong absorption range from 300 to 700 nm, which was favorable to enhance the photocurrent response. As we all know, the photoluminescence of semiconductors is generated during the recombination process of photogenerated electron-hole pairs, and the photoluminescence intensity is related to the recombination rate. A high recombination rate can be usually accompanied by the strong photoluminescence intensity. Thus, the charge transfer phenomenon of semiconductors could be clarified by analyzing the PL spectra. As shown in Fig. 1F (red lines), the PL spectra of pure g-C₃N₄ (curve *a'*) and g-C₃N₄/CdS:Mn (curve *b'*) were measured at an excitation wavelength of 365 nm. The PL spectra of pure g-C₃N₄ showed a strong PL emission peak around 440 nm, and the PL intensity of g-C₃N₄ was higher than its composites, indicating that g-C₃N₄/CdS:Mn had an efficient and higher rate of separation of electron-hole pairs compared with pure g-C₃N₄.

3.2. PEC response of g-C₃N₄/CdS:Mn-modified electrode

As mentioned above, the g-C₃N₄/CdS:Mn composites had an enhanced light absorbance and a high electron-hole pairs separation efficiency. To assess the PEC performance of the as-synthesized materials, the same-amount photoactive materials were dropped onto the cleaned electrode, and then recorded their photocurrent responses under light. As shown in Fig. 2A, the photocurrent response of pure g-C₃N₄ (curve *a*) was weak, which was related to its low photon-to-electron conversion efficiency and wide band gap (Fan et al., 2016). For comparison, CdS:Mn QDs (curve *b*) showed a higher photocurrent than that of g-C₃N₄ due to its narrow band gap. Besides, the presence of manganese ions in the composites could promote the transfer of photogenerated electrons. After the combination of g-C₃N₄ and CdS:Mn QDs, the composites could enhance the photocurrent response of about 55% (compared with that of CdS:Mn-modified electrode). The generation mechanism of photocurrent is shown in Scheme 1A, the photosensitive material could produce photogenerated electron-hole pairs under the irradiation of light. Because of the well-matched band positions of the two photoactive materials, photo-excited electrons in the conduction band (CB) of g-C₃N₄ could be more easily transferred to the CB of CdS:Mn, then holes are transferred concurrently from the valence band (VB) of CdS:Mn to the VB of g-C₃N₄, which leading to an improved photocurrent.

179

180 **Fig. 2.** (A) Photocurrent responses of (a) g-C₃N₄/GCE, (b) CdS:Mn/GCE, (c) g-C₃N₄/CdS:Mn/GCE; (B) Influence
 181 of CdS:Mn concentration on the photocurrent response of g-C₃N₄/CdS:Mn-modified electrode (note: curve a, 0.5
 182 mg mL⁻¹; curve b, 1 mg mL⁻¹; curve c, 1.5 mg mL⁻¹; curve d, 2.0 mg mL⁻¹; curve e, 2.5 mg mL⁻¹); (C) Influence of
 183 potential on PEC response; (D) The stability of photocurrent response of g-C₃N₄/CdS:Mn/GCE.

184 Also, we studied the photocurrent response of the composites obtained by mixing different
 185 amounts of the two materials. In this work, the concentration of g-C₃N₄ was always maintained at
 186 1.0 mg mL⁻¹ in the composite solutions, while the concentration of CdS:Mn was varied from 0.5 to
 187 2.5 mg mL⁻¹. As indicated in Fig. 2B, the photocurrent of the g-C₃N₄/CdS:Mn-modified electrode
 188 was also enhanced correspondingly (in the range of 0.5 to 2.0 mg mL⁻¹, curves a~d) with the
 189 increase of the CdS:Mn concentration in the composites, indicating that the increase of the amount
 190 of CdS:Mn in the composites was conducive for the transfer of the photogenerated electrons and
 191 holes. Moreover, when the concentration of CdS:Mn in the composites was higher than 2.0 mg mL⁻¹,
 192 the photocurrent decreases sharply (curve e). This might be associated with excessive amounts of
 193 CdS:Mn in the composites, which would restrain the transfer of the electrons and holes. Hence, 2.0
 194 mg mL⁻¹ CdS:Mn was selected as the optimum concentration for the construction of the PEC

195 sensor.

196 The anodic potential applied to the electrodes also has an effect on the photocurrent response. In
197 general, a large anodic potential could impel more photogenerated electrons to the counter electrode
198 and inhibit the recombination of electron-hole pairs more effectively (Liu et al. 2015). As depicted
199 in Fig. 2C, the photocurrent response of g-C₃N₄/CdS:Mn-modified electrode gradually increased as
200 the applied anodic potential varied from 0.2 to 0.8 V. Because of high applied potential is bad for
201 biomolecule samples and electrodes (Zhang et al. 2016), thus, 0.6 V was chosen for the following
202 PEC sensing. Fig. 2D showed the good stability of the g-C₃N₄/CdS:Mn-modified electrode under a
203 long time of PEC measurement.

204 3.3. Characteristics of g-C₃N₄/CdS:Mn-based PEC sensing platform

205 In this work, the Ab₁ was immobilized on the electrode by glutaraldehyde *via* the cross-linking
206 reaction. Colloidal gold nanoparticles were labeled with S₀ and Ab₂ (S₀-AuNP-Ab₂) for the
207 detection of antigens. In the presence of target PSA, the immobilized Ab₁, PSA and S₀-AuNP-Ab₂
208 would generate a sandwiched immunocomplex. During the process, S₀-AuNP-Ab₂ was successfully
209 introduced into the electrode. In our design, the sequences of S₀ are exactly the same as 15 bases at
210 the 3' end of S₂, and are fully complementary to the 15 bases at the 3' end of S₁. The 15 bases in the
211 middle of S₁ are fully complementary with the 15 bases at the 5' end of S₂, and the beginning 20
212 bases at the 5' end of S₁ are the binding region for hemin to form the mimetic enzyme. S₁ and S₂
213 were used for the concatamer reaction, a nicked double-helix strand is formed by alternating these
214 two DNA pre-designed strands in sequence, and the amount of mimetic enzyme is related to the
215 length of the nicked strand. The formed DNAzyme could effectually catalyze the oxidation of 4-CN
216 in the presence of H₂O₂ to produce an insoluble/insulating product (*i.e.*, benzo-4-chlorohexadienone
217 precipitate, the catalytic product inhibits the transfer of electrons on the electrode, resulting in the
218 reduction of photocurrent). Hence, the formation of the DNAzyme is crucial to the results of the
219 experiment. As mentioned above, a sandwich structure could be formed in the presence of PSA. S₀
220 on the gold nanoparticles, which acted as initiator strand, and the presence of S₀ ensures that the
221 long nicked chains formed by S₁ and S₂ could be bound to the S₀-AuNPs-Ab₂ bioconjugates.
222 Without PSA, the S₀-AuNP-Ab₂ cannot be conjugated onto the electrode, and it also prevents the
223 long chains from being fixed to the electrode, thus exhibiting a low signal. By monitoring the

change of the photocurrent, we can get the concentration of PSA in the samples.

To further verify our design, the S_0 -AuNPs-Ab₂ bioconjugates were characterized by using gel electrophoresis. Fig. 3A gives the representative gel electrophoresis of multifarious components. As seen from lanes 2 and 3, two electrophoretic bands were very close, which was consistent with our design. Due to the fact that S_0 -AuNP-Ab₂ could not migrate during the electrophoresis, a strong electrophoretic band was found at the beginning of lane 4. After mixing 1.0 μ M S_1 and 1.0 μ M S_2 , an electrophoresis band with a quite wide range appeared on lane 5, indicating that the formation of nicked chains as a result of their self-hybridization reaction. When the S_0 -AuNP-Ab₂ solution was incubated with 1.0 μ M S_1 and 1.0 μ M S_2 , a strong electrophoresis band was seen at the beginning of lane 6, and a wide electrophoresis band with a weak intensity was also present on lane 6. The presence of wide electrophoresis band indicated the formation of nicked chains. Compare with lane 5, the intensity of the electrophoresis band on lane 6 was weaker than that of 5, which was related to the small amount of DNA on lane 6. These results indicated that the DNA concatamers were successfully bound to the S_0 -AuNP-Ab₂. Moreover, the UV-visible absorption spectroscopy was used to characterize the successful synthesis of S_0 -AuNP-Ab₂. As seen from Fig. 3B, the absorbance of Ab₂ antibody (curve *a*) was low, which was related to its low concentration. The spectra of DNA (S_0 , curve *b*) showed a characteristic absorption peak at 260 nm, and the maximum absorption peak of curve *c* was at 518 nm, which belongs to the characteristic absorption peak of pure gold nanoparticles. After gold nanoparticles interacted with DNA and Ab₂ antibodies, the position of absorption peak was shifted from 518 nm to 524 nm (curve *d*), indicating the formation of AuNPs-Ab₂. Besides, another absorption peak at 260 nm (curve *d*) indicates that DNA was successfully modified on gold nanoparticles. Thus, the presence of these two absorption peaks indicated the successful synthesis of S_0 -AuNP-Ab₂. The results of gel electrophoresis were consistent with the results of UV-visible absorption spectra.

Next, the electrochemical impedance spectroscopy (EIS) was employed to analyze the interfacial properties of the modified electrode in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ (as the redox probe) containing 0.1 M KCl (Fig. 3C). These EIS data were fitted to a Randles equivalent circuit (Fig. 3C, inset), which contains electrolyte resistance (R_s), the lipid bilayer capacitance (C_{dl}), charge transfer resistance (R_{et}) and Warburg element (Z_w). Typically, the semicircular diameter, which appears in the Nyquist plot of EIS, is equal to the value of electron transfer resistance (R_{et}). As shown in Fig. 3C, the R_{et} value

254 of a clean GCE (curve *a*) was very small, after coating g-C₃N₄/CdS:Mn on electrode surface, R_{et}
 255 value increased significantly (curve *b*), which may be due to the introduction of g-C₃N₄/CdS:Mn
 256 hindered the electron transfer on the electrode. Then, the modified electrodes were incubated with
 257 BSA/Ab₁/CS, PSA & S₀-AuNPs-Ab₂, DNA (S₁ and S₂) and hemin in turn, and the R_{et} value
 258 gradually increased (curves *c-f*). The reasons for the increase in the resistance value (curves *c-d*)
 259 were owing to the introduction of proteins and the formation of immune complexes, indicating the
 260 poor conductivity of the biomacromolecule. After the S₁ and S₂ were assembled onto the surface of
 261 the S₀-AuNP-Ab₂, the R_{et} was significantly increased, which was due to the repulsion between
 262 negatively charged DNA and negatively charged ferricyanide. After adding hemin to form
 263 DNAzyme, the change of resistance value was not obvious. After the resulting electrode was
 264 immersed in a mixed solution containing H₂O₂ and 4-CN, the R_{et} was found to be greatly increased.
 265 The results may be attributed to the fact that formed hemin/G-quadruplex could catalyze 4-CN to
 266 produce an insoluble product, which covers the surface of the immunosensor and seriously hinders
 267 the transfer of electrons between the electrolyte solution and the electrode.

268 Further, we also investigated the photocurrent responses of the electrode after each modification
 269 steps. As depicted in Fig. 3D, the g-C₃N₄/CdS:Mn-modified electrode exhibited a high photocurrent
 270 response (curve *a*), which was beneficial to the subsequent modification steps and DNAzyme-based
 271 inhibition analysis. With the introduction of CS (curve *b*), BSA/Ab₁ (curve *c*), PSA (1.0 ng mL⁻¹) +
 272 S₀-AuNPs-Ab₂ (curve *d*), DNA (S₁ and S₂, curve *e*) and hemin (curve *f*), the photocurrent responses
 273 were gradually reduced, these results were consistent with the EIS results we obtained before. In
 274 addition, the photocurrent was largely reduced after the modified electrode was incubated with a
 275 mixed solution containing 4-CN and H₂O₂ (curve *g*). The reasons for the photocurrent decrease
 276 could be explained as follows: (i) the substances inhibit the transfer of electrons at the solid-liquid
 277 interface, which was the same as EIS. (ii) These substances obscure a portion of the excitation light
 278 such that the reduction of the light irradiated onto the photosensitive material, the decrease of light
 279 eventually results in a reduction in the number of photogenerated electron-hole pairs.

280

281 **Fig. 3.** (A) Gel electrophoresis (lane 1: DNA marker; lane 2: 1.0 μM S_1 ; lane 3: 1.0 μM S_2 ; lane 4: S_0 -AuNP- Ab_2 ;
 282 lane 5: 1.0 μM S_1 + 1.0 μM S_2 ; lane 6: 1.0 μM S_1 + 1.0 μM S_2 + S_0 -AuNP- Ab_2); (B) UV-vis absorption spectra of
 283 (a) Ab_2 antibody, (b) S_0 , (c) Au NP, and (d) S_0 -AuNP- Ab_2 ; (C) EIS spectra of (a) GCE, (b) $\text{g-C}_3\text{N}_4/\text{CdS:Mn}/\text{GCE}$,
 284 (c) sensor b + BSA/ Ab_1 /CS (1.0 ng mL^{-1} target PSA used as an example), (d) sensor c after reaction with PSA and
 285 S_0 -AuNP- Ab_2 , (e) sensor c after reaction with S_1 and S_2 , (f) sensor d + hemin, and (g) sensor e + 4-CN + H_2O_2 in
 286 0.1 M KCl containing 5.0 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ (inset: equivalent circuit); (D) Photocurrent responses of (a)
 287 $\text{g-C}_3\text{N}_4/\text{CdS:Mn}/\text{GCE}$, (b) sensor a + CS, (c) sensor b + BSA/ Ab_1 , (d) sensor c after reaction with PSA and
 288 S_0 -AuNP- Ab_2 , (e) sensor d after reaction with S_1 and S_2 , (f) sensor e + hemin, and (g) sensor f + 4-CN + H_2O_2 .

289 3.4. Optimization of experimental conditions

290 To obtain the best analytical performance of the PEC sensor, several experiment parameters were
 291 investigated. The results were described in Fig. S1 of the Supporting Information. An incubation
 292 time of 40 min, hybridization time of 100 min and catalytic deposition time of 40 min were chosen
 293 as the best experimental conditions for determination of PSA.

294

295 **Fig. 4.** (A) Effect of different-concentration PSA standards on the photocurrent responses; (B) the corresponding
 296 calibration curve of the PEC immunoassay and (C) the specificity of the proposed PEC immunoassay to target
 297 PSA (10 ng mL⁻¹) by comparing it to other interfering substances: CEA (10 ng mL⁻¹), AFP (10 ng mL⁻¹), CA 15-3
 298 (10 U mL⁻¹) and the mixed-samples.

299 3.5. Analytical performance of PEC immunosensor for PSA detection

300 Under optimal conditions, the analytical properties of the immunosensor were evaluated with
 301 PSA standards by using the PEC detection method. The photocurrent decreased with the increase of
 302 PSA concentration in the samples (Fig. 4A). Moreover, the ratio value ($\Delta I/I_0$, $\Delta I = I_0 - I$, where I and
 303 I_0 represent the photocurrent of the immunosensor in the presence and absence of target PSA,
 304 respectively) increased linearly with the logarithm of PSA concentration in the range from 0.01 to
 305 20 ng mL⁻¹ (Fig. 4B). The linear regression equation between ΔI and the logarithm of PSA level
 306 was fitted to $\Delta I (\mu A) = 0.2558 \times \lg C_{[PSA]} - 0.1073$ (pg mL⁻¹, $R^2 = 0.9962$, $n = 24$) with a detection
 307 limit (LOD) of 3.8 pg mL⁻¹ at the signal-to-noise ratio of 3. Obviously, the LOD of our system was
 308 comparable with other PSA detection schemes (Table S1 in Supporting Information). Furthermore,
 309 the obtained LOD by the immunosensor was also lower than that of commercialized human PSA
 310 ELISA kit purchased from Sigma-Aldrich (LOD: 8.0 pg mL⁻¹). All data were obtained from three
 311 parallel experiments, and maximum relative standard deviation (RSD) was 8.2%. Also, we
 312 evaluated the sensor-to-sensor reproducibility toward three different concentrations (0.05, 1.0, 10
 313 ng mL⁻¹ PSA), and the RSDs were 9.1%, 6.6%, and 8.0% ($n = 3$), respectively, suggesting good
 314 reproducibility.

315 To evaluate the specificity of PEC immunosensor toward target PSA, other biomarkers in human
 316 serum such as carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), cancer antigen 15-3 (CA

15-3) were chosen as the interference proteins to examine the anti-interference ability of this system. As depicted in Fig. 4C, the photocurrents toward interfering proteins were slightly higher or lower than that of the background signal (*i.e.*, blank sample), while the response to target PSA was very strong. Moreover, the photocurrent of the mixture containing PSA was approximately the same as pure PSA standard, indicating that the coexistence of the PSA with the interfering substances did not have a significant effect on the photocurrent of the immunosensor. These results illustrated the high specificity and anti-interference ability of PEC immunosensor.

To further investigate the application potential of PEC immunosensor in human serum samples, six human samples obtained from the local hospital were measured by using PEC immunosensor and commercialized human PSA ELISA kit (Sigma), respectively. The results are listed in Table 1. Statistical comparison between two methods was performed by using a *t* test for comparison of means preceded by the application of a *t*-test. At the 0.05 significance level, the t_{exp} values were less than t_{crit} ($t_{\text{crit}[0.05,4]} = 2.77$). Therefore, there is no significant difference between two methods. Based on the above experimental results, this method could be valuable for the detection of PSA in clinical diagnostics as an optional scheme.

Table 1. Comparison of the assayed results by using PEC immunosensor and PSA ELISA kit

Sample no.	Method; Concentration (mean $\pm$ SD, $n = 3$, ng mL ⁻¹)		t_{exp}
	Found by immunosensor	Found by ELISA	
1	1.94 $\pm$ 0.12	1.83 $\pm$ 0.10	1.22
2	0.032 $\pm$ 0.003	0.030 $\pm$ 0.002	0.96
3	3.89 $\pm$ 0.17	4.17 $\pm$ 0.22	1.74
4	13.42 $\pm$ 1.11	12.34 $\pm$ 1.21	1.14
5	0.82 $\pm$ 0.07	0.75 $\pm$ 0.03	1.59
6	8.57 $\pm$ 0.66	9.25 $\pm$ 0.77	1.16

4. Conclusions

In summary, we have developed a new photoelectrochemical assay for highly sensitive detection of PSA by using g-C₃N₄/CdS:Mn nanohybrids as the photoactive materials, based on DNAzyme concatamers and enzymatic biocatalytic precipitation. Compared with single photosensitive material alone, the analytical performance of PCE immunosensor could be improved by using g-C₃N₄ and CdS:Mn QDs-based nanocomposites. Moreover, the PEC immunosensing platform did not require the participation of natural enzymes, since hemin could bind to the dendritic single-stranded DNA

on DNA concatamers to form peroxidase-mimicking DNAzyme, which catalyzed the precipitation reaction of 4-CN, resulting in the reduction of photocurrent. In addition, the immunoassay did not contain the sophisticated fabrication, and showed a wide linear range with an acceptable precision and accuracy, thereby presenting an optional scheme in the field of clinical diagnostics.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Grant nos.: 21675029 and 21475025), the National Science Foundation of Fujian Province (Grant no.: 2014J07001), and the Program for Changjiang Scholars and Innovative Research Team in University (Grant no.: IRT15R11).

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