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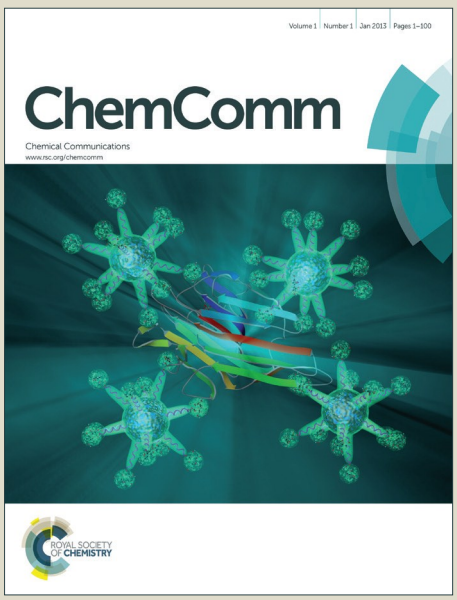




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ARTICLE TYPE

# An electrochemical peptide cleavage-based biosensor for matrix metalloproteinase-2 detection with exonuclease III-assisted cycling signal amplification

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**In this work, an electrochemical peptide biosensor was developed for matrix metalloproteinase-2 (MMP-2) detection by converting a peptide cleavage event into DNA detection with exonuclease III (Exo III)-assisted cycling signal amplification.**

Matrix metalloproteinase 2 (MMP-2), a zinc-dependent proteinase capable of degrading extracellular matrix proteins, has been found to be closely associated with pathological progression of cancer[1,2]. Therefore, the quantification of MMP-2 is significant for disease diagnosis and therapy. Up to now, the most widely used methods for MMP-2 detection are based on immunoassays, including enzyme-linked immunosorbent assay (ELISA)[3] and surface plasmon resonance (SPR) immunosensing[4]. Although these immunoassays exhibit promising sensitivity and specificity, they utilize antibodies as recognition elements with some drawbacks, for instance, the requirement of producing high-quality antibodies, the difficulty in site-specific bioconjugation and maintaining the initial activity[5-7].

Recently, a short peptide, which is selected using the phage display technique, has been developed as a more compelling choice than an antibody in protein recognition due to its great advantages, such as simplicity, accessibility, chemical definition, cost effectiveness, etc[8,9]. Since Heller *et al.* has reported that a specific peptide with the amino acid sequence of PLGVR can be specifically hydrolyzed and cleaved by MMP-2[10]. Then several peptide-based methods including fluorescence assay[11], bioluminescence assay[12], and electrochemical assays[13] have been developed for MMP-2 detection. Among those techniques, the electrochemical method has attracted considerable attention due to its distinct advantages such as speed, sensitivity, and convenience[14]. Lin and Chen groups have developed the electrochemical methods based on target induced cleavage of substrate peptides to analyze MMP-2[15,16]. However, these biosensors are signal-off assays suffering from problems of limited signal output and "false positive" results. In addition, there are no effective signal amplification strategies, leading to an intrinsic limitation on sensitivity. Therefore, the analyzing of MMP-2 with low concentration still faces great challenges.

Currently, the approach based on combining protein analysis with DNA amplification strategies has been developed and recognized as a powerful tool to improve the sensitivity for protein detection. And traditional DNA signal amplification strategies include polymerase chain reaction (PCR)[17,18], rolling circle amplification (RCA)[19,20] could realize sensitive detection for target but are accompanied with some drawbacks, for instance, the requirements of precise temperature control, a complex sets of primers, and professional handling procedures with high costs. Recently, enzyme-assisted cycling amplification strategy[21-23] holds great promise in signal amplification process due to its isothermal reaction, easy operation and low cost. For example, exonuclease III (Exo III), a sequence-independent nuclease that could specifically hydrolyze mononucleotide from its blunt or the recessed 3′ termini to 5′ termini in duplex DNA. This feature of selective nucleotides digestion for Exo III may be utilized to "recycle" target or substance related with target, which could significantly enhance the detectable signal, leading to a high sensitivity.

Herein, we converted the peptide cleavage event into DNA detection, followed by Exo III-assisted cycling signal amplification for sensitive analysis of MMP-2. A peptide-functionalized magnetic polystyrene microsphere (PSC-peptide) was firstly coupled with $DNA_1$-modified gold nanoparticles (AuNPs-$DNA_1$) to act as a recognition probe (PSC-peptide-AuNPs-$DNA_1$). Since the target MMP-2 can specifically cleave the peptide in recognition probe, the AuNPs-$DNA_1$ was quantitatively released from magnetic microsphere in the presence of MMP-2, which further was used to hybridize with the methylene blue-labeled $DNA_2$ (MB-$DNA_2$) to form duplex DNA with a recessed 3′terminus at the $DNA_2$. With the introduction of Exo III, MB-$DNA_2$ existed in duplex DNA was selectively digested and released the free electroactive substance MB. During this reaction, AuNPs-$DNA_1$ was simultaneously dissociated from the digested $DNA_2$ and could be used to hybridize with other MB-$DNA_2$, starting a new cycle of MB-$DNA_2$ digestion. In this way, a single AuNPs-$DNA_1$ could trigger the digestion of multiple MB-$DNA_2$ into numerous free MB molecules, which could be captured by cucurbit[7]uril (CB[7]) *via* host-guest interaction to produce noticeable electrochemical signal. By monitoring the changed electrochemical signal, MMP-2 could be determined quantitatively. Moreover, the employment of electrodeposition of AuNPs（depAu) functionalized electrode as sensor platform can not only effectively immobilize of CB[7] for capturing free MB, but also facilitate electron transfer from

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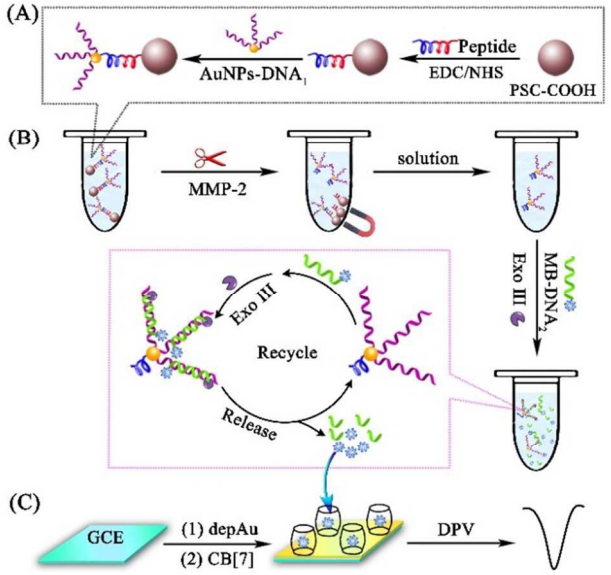
MB to the electrode surface, the developed biosensor exhibited excellent performance for the electrochemical detection of MMP-2 with high sensitivity and specificity. The schematic diagram of a stepwise assembled procedure of the biosensor and the electrochemical detection principle are shown in Scheme 1(See ESI for more experimental details).




**Scheme 1** Schematic illustration of the electrochemical peptide cleavage-based method for MMP-2 detection: (A) the preparation of the PSC-peptide-AuNPs-$DNA_1$ biocomplex; (B) the magnetic separation and Exo III-assisted cycling process; (C) the preparation of functionalized electrode and electrochemical detection of MMP-2.

The cyclic voltammetric (CV) measurement has been employed to characterize the assembly steps of the sensing interface. As can be seen in Fig. 1A, a well-defined redox peak of $[Fe(CN)_6]^{3-/4-}$ was observed at bare electrode (curve a). When depAu was modified on the electrode surface, the redox peak current increased obviously (curve b), which was attributed to the superior conductivity of depAu. Subsequently, with the introduction of CB[7], a decreased peak current was acquired (curve c), indicating that CB[7] was successfully assembled on the modified electrode surface.

Electrochemical impedance spectroscopy (EIS) was further employed to characterize the electrochemical behaviors of the modified electrode at different stages in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. As can be seen in Fig. 1B, the bare GCE showed a very small semicircle (curve a), indicating a low transfer resistance. After the electrode was modified with depAu, a smaller semicircle (curve b) was obtained owing to the conductivity of depAu. When CB[7] was immobilized on the modified electrode, the resistance increased slightly (curve c), which was attributed to the non-electroactive property of CB[7]. The results indicated that the sensing interface was effectively constructed.

To evaluate analytical performance of the proposal platform, we performed the designed strategy with different concentrations of MMP-2 and the electrochemical responses were recorded by DPV. From Fig. 2A we could see that, the electrochemical signal increased with increasing concentration of MMP-2. Fig. 2B displayed the corresponding calibration curve of developed biosensor, where the electrochemical signals were proportional to the logarithm concentration of MMP-2 in the range from 0.5 $pg \cdot mL^{-1}$ to 50 $ng \cdot mL^{-1}$ with a detection limit of 0.15 $pg \cdot mL^{-1}$. The regression equation was $I$ = - 227.01 $lgc_{MMP-2}$ - 4276.8 with a correlation coefficient of 0.9982. We also compared the performance of the present work with other reported methods. As shown in Table S1 (see ESI), our method had a broad linear range, quite low detection limit and high sensitivity, clearly demonstrated that our proposed strategy was efficient for electrochemical detection of target MMP-2.

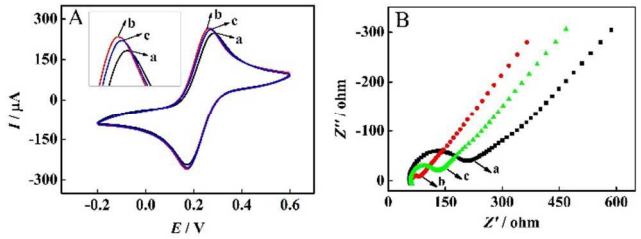



**Fig. 1** The electrochemical characterization of the stepwise modified electrodes: CV (A) and EIS (B) of bare GCE (a); depAu/GCE (b); CB[7]/depAu/GCE in 0.10 M PBS (pH 7.0) containing 5 mM $[Fe(CN)_6]^{3-/4-}$.

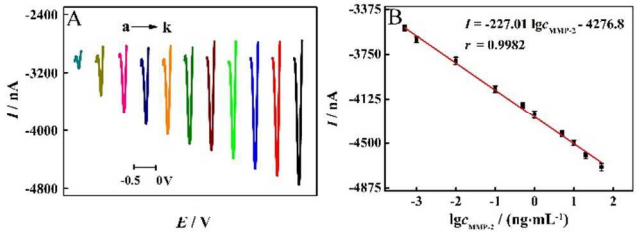



**Fig. 2** (A) The DPV responses of proposed biosensor with different MMP-2 concentrations (from a to k: 0. 0.0005, 0.001, 0.01, 0.1, 0.5, 1, 5, 10, 20, 50 $ng \cdot mL^{-1}$), detection buffer: PBS (pH 7.0) and (B) the calibration plot of current intensity *vs*. $lgc_{MMP-2}$.

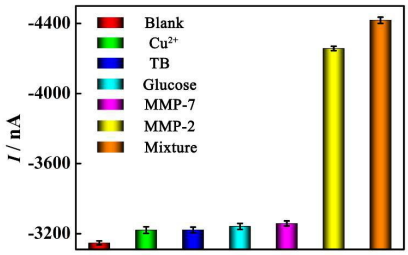



**Fig. 3** Specificity study of the proposed biosensor for MMP-2 detection.

Selectivity is an important criterion for a successful assay system in protein detection. In order to evaluate the specificity of the electrochemical biosensor, some other interferences such as $Cu^{2+}$, thrombin (TB), glucose and matrix metalloproteinase-7 (MMP-7) were assayed under the same experimental conditions. As shown in Fig 3, no significant change of the current response could be observed in the detection of $Cu^{2+}$ (10 $ng \cdot mL^{-1}$), TB (10 $ng \cdot mL^{-1}$), glucose (10 $ng \cdot mL^{-1}$) and MMP-7 (10 $ng \cdot mL^{-1}$) in comparison with the blank test. On the contrary, a high current response was obtained after incubation with the target MMP-2 (1 $ng \cdot mL^{-1}$). Meanwhile, when 1 $ng \cdot mL^{-1}$ MMP-2 coexisted with

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the above four interferences (10 ng·mL$^{-1}$), no apparent signal change existed compared with the case of only MMP-2. All these results indicated that the proposed biosensor has a good specificity for MMP-2.

The reproducibility of the biosensor was evaluated by analysis of the same concentration of MMP-2 (20 ng·mL$^{-1}$) using four electrodes under the same conditions. Closely electrochemical responses and a relative standard deviation (RSD) of 5.90% were obtained. This result revealed the acceptable reproducibility of the constructed biosensor. Besides, the stability of the designed biosensor was investigated by storing the proposed electrode at 4 °C and measuring every 6 days. After a longer storage for 18 days, the electrochemical response retained 92.4% of the initial current response. This result demonstrated that the prepared biosensor exhibited satisfactory stability.

To monitor the reliability of the developed biosensor, recovery experiments were performed by adding various concentrations of MMP-2 into the 20-fold diluted healthy human serum (obtained from the Third Military Medical University of Chongqing, China). As indicated from Table S2 (See ESI), the recoveries ranged from 94.06% to 102.32% with relative standard deviation values (RSD) ranging from 2.31% to 5.34%. High recoveries and acceptable RSD indicated the potentiality of this method for MMP-2 detection in clinical applications.

In our method, the peptide cleavage event was translated into DNA detection and coupled with Exo III-assisted cycling for signal amplification, providing a simple, sensitive strategy for MMP-2 detection. Compared with other protocols, our method has four significant advantages: (1) the ''signal-on'' assay could minimize false positive results which were caused by electrode stripping or fouling[24]; (2) the approach by combining protein analysis with a DNA signal amplification strategy significantly enhanced the detection sensitivity[25]; (3) the adoption of the CB[7]/depAu modified electrode may greatly promote the signal acquisition for electrochemical detection, which not only takes advantage of good conductivity and large surface-to-volume ratio of depAu but also profits from the strong and selective supramolecular recognition ability of CB[7] to MB[26]; (4) this method was based on the target-induced cleavage specific peptide for MMP-2 detection, so it could be extended for the determination of other proteins that display similar property[27].

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## Notes and references

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† Electronic Supplementary Information (ESI) available: [Experimental section; the preparation of bioconjugated magnetic nanoprobe and the preparation of modified electrode; optimization of the experimental conditions]. See DOI:10.1039/b000000x/

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