ACS APPLIED MATERIALS & INTERFACES

Article

DNA Enzyme Decorated DNA Nanoladders as Enhancer for Peptide Cleavage-Based Electrochemical Biosensor

Beibei Kou, Li Zhang, Hua Xie, Ding Wang, Yali Yuan, Yaqin Chai, and Ruo Yuan

ACS Appl. Mater. Interfaces, **Just Accepted Manuscript** • DOI: 10.1021/acsami.6b07017 • Publication Date (Web): 17 Aug 2016

ACS Publications

ACS Applied Materials & Interfaces is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

DNA Enzyme Decorated DNA Nanoladders as Enhancer for Peptide Cleavage-Based Electrochemical Biosensor

Bei-Bei Kou, Li Zhang, Hua Xie, Ding Wang, Ya-Li Yuan, Ya-Qin Chai, Ruo Yuan**

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ABSTRACT: Herein, we developed a label-free electrochemical biosensor for sensitive detection of matrix metalloproteinase-7 (MMP-7) based on DNA enzyme decorated DNA nanoladders as enhancer. A peptide and single-stranded DNA S_1 modified platinum nanoparticles (P_1-PtNPs-S_1) which served as recognition nanoprobes were firstly immobilized on electrode. When target MMP-7 specifically recognized and cleaved the peptide, the PtNPs-S_1 bioconjugates were successfully released from electrode. The remaining S_1 on electrode then hybridized with ssDNA1 (I_1) and ssDNA2 (I_2), which could synchronously trigger two hybridization chain reactions (HCRs), resulting in the in-situ formation of DNA nanoladders. The desired DNA nanoladders not only were employed as ideal nanocarriers for enzyme loading, but also maintained its catalytic activity. With the help of hydrogen peroxide (H_2O_2),

*Corresponding authors at: Tel.: +86-23-68252277, fax: +86-23-68253172.

E-mail addresses: y198688@swu.edu.cn (YL. Yuan); yuanruo@swu.edu.cn (R. Yuan).

manganese porphyrin (MnPP) with peroxidase-like activity accelerated the 4-chloro-1-naphthol (4-CN) oxidation with generation of insoluble precipitation on electrode, causing a very low differential pulse voltammetry (DPV) signal for quantitative determination of MMP-7. Under optimal conditions, the developed biosensor exhibited a wide linear ranging from 0.2 pg/mL to 20 ng/mL and the detection limit was 0.05 pg/mL. This work successfully realized the combination of DNA signal amplification technique with artificial mimetic enzyme catalyzed precipitation reaction in peptide cleavage-based protein detection, offering a promising avenue for the detection of other proteases.

KEYWORDS: Matrix metalloproteinases-7; DNA nanoladders; Manganese porphyrin (MnPP); Signal amplification; 4-chloro-1-naphthol (4-CN)

1. Introduction

Matrix metalloproteinase-7 (MMP-7) is able to degrade a great diversity of extracellular matrix (ECM) consisting of type IV collagen, fibronectin as well as several non-ECM molecules.[1,2,3] Studies have shown that MMP-7 is almost closely linked to many of fatal diseases such as cancer, which thus is considered as an important biomarker for a great many tumors.[4,5] Until now, a variety of approaches, including fluorescence resonance energy transfer (FRET)-based assays [6], enzyme-linked immunosorbent assays (ELISA)[7], have been developed. However, there remain some drawbacks including expensive, fussy, low sensitivities.[8] A

simple and sensitive method is thus urgently needed for detection of MMP-7 in diagnostics.

Electrochemical biosensors with the advantages of low cost, easy operation, high sensitivity and good selectivity can meet the above mentioned requirement to a certain extent.[9] To further improve the sensitivity, natural protein assisted enzyme amplification are commonly used, which however still suffered from difficult storage and fussy labeling progress that restrict their further applications.[10] In comparison, the artificial mimetic enzymes assisted enzyme amplification[11] achieved the relatively high chemical stability and simple synthesis.[12] More importantly, the artificial mimetic enzymes like porphyrin exhibit superior catalytic activity and can be immobilized accompanied with the formation of DNA nanowires without fussy labeling progress. For instance, manganese porphyrin (MnPP) with excellent peroxidase-like activity[13] can be intercalated into the grooves of negatively charged double-stranded DNA nanowires without any label steps for signal amplification[14]. Therefore, the number of DNA nanowire is crucial for MnPP associated amplifying system. Traditional methods, such as rolling circle amplification (RCA)[15,16], hybridization chain reaction (HCR)[17,18], ligase chain reaction (LCR)[19] and polymerase chain reaction (PCR)[20] can usually obtain just one corresponding DNA nanowire triggered by each copy of the initiators, and thus limited the immobilizing amount of MnPP, resulting in a low catalytic efficiency. To overcome this drawback, an initiator triggers simultaneously multiple assembly processes is required. Zhou et

al. reported that one initiator could synchronously trigger two HCRs through self-assembly processes for forming DNA nanoladders.[21] Since DNA nanoladders can produce multiple DNA nanowires, substantial MnPP with high catalytic activity can be loaded, providing a simple and effective approach for sensitive protein assay.

Herein, we proposed a "signal-on" peptide cleavage-based biosensor for sensitive detection of MMP-7, coupling DNA nanoladders with artificial mimetic enzyme MnPP catalyzed precipitation for signal amplification. As shown in Scheme 1, a peptide and ssDNA S_1 functionalized platinum nanoparticles (P_1-PtNPs-S_1) was acted as recognition nanoprobe. The peptide with the amino acid sequence (NH_2-KKKRPLALWRSCCC-SH) possessed a MMP-7 cleavage site. With the introduction of target MMP-7, some PtNPs-S_1 bioconjugates containing peptide fragments were removed from the electrode. The remaining S_1 on electrode then provided the binding sites to immobilize I_1 and I_2 to trigger the generation of DNA nanoladders. To assemble nanoladders, the motifs (M_1 and M_2) consisted of three domains: a single-duplex helix and two flanking tail-hairpin structures. Single stranded DNA_1 (I_1) and I_2 paired up to form identical single-duplex helix, and they were initiators that triggered two HCRs at the same time. The formed DNA nanoladders could effectively increase the load amount of MnPP. Herein, MnPP was utilized as catalyst to electrocatalyze the substrate 4-CN to generate insoluble precipitation, and thus a relatively small electrochemical signal was obtained.

Therefore, the proposed biosensor could achieve the highly sensitive and selective quantitative analysis for MMP-7.

2. Experimental

2.1 Chemicals and apparatus

Manganese porphyrin (MnPP), tris (2-carboxyethyl) phosphine (TCEP), gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), chloroplatinic acid (H_2PtCl_6), 4-chloro-1-naphthol (4-CN) and mercaptohexanol (MCH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid (AA) was received from Chemical Group Co., Ltd (Chongqing Chuandong). Matrix metalloproteinase-7 (MMP-7) was provided by Sino Biological Inc. (Beijing, China). The peptide P_1 (NH_2-KKKRPLALWRSCCC-SH) was purchased from Science Peptide Biological Technology Co., Ltd (Shanghai, China).

The synthetic DNA oligonucleotides were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China) with sequences as follows:

S_1: 5'-AAA AAA AAA AGC GCT AGA TAA CAT AGT GTA T-3'

For nanoladders:

M_1: 5'-TAG AGA GCG CTC TCT AGT TGA AGG TGG ATG AGT GGA GTG AAG ACC ATC CAA CTT CAC TCC ACT CAT CC-3'

M_2: 5'-CTT CAC TCC ACT CAT CCA CCT TCA ACG GAT GAG TGG AGT GAA GTT GGA TGG TTA GAG AGC GCT CTC TA-3'

I_1: 5'-CTT CAC TCC ACT CAT CCA CCT TCA ACT TTT TTT AGA GAG CGC TCT CTA-3'

I_2: 5'-CTT CAC TCC ACT CAT CCA CCT TCA ACT TTT TTT AGA GAG CGC TCT CTA GAT CTA TTG TAT CAC-3'

Tris-HCl-EDTA-Mg^{2+} buffer[21] was served as DNA oligonucleotides buffer. Phosphate buffered solution (PBS, pH 7.4, 0.1 M)[22] was used as working buffer.

2.2. Apparatus and Measurements

The CHI 660D electrochemical workstation was used to conduct all electrochemical measurements.[23] The images of different modified electrodes were performed by atomic force microscopy (AFM, Nanoscope V Multi-mode 8, Bruker Company, Santa Barbara, USA) and scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). Gels images were obtained by a Bio-Rad imaging system (Hercules, CA, U.S.A.).

2.3 Preparation of Pt nanoparticles (PtNPs) and P_1-PtNPs-S_1 bioconjugates

The PtNPs were prepared according to ref. 24. Fig. 1 showed a typical SEM image of PtNPs which exhibited well-formed spherical structure, and their distribution was uniform. In addition, the average size of PtNPs was 50 nm. The P_1-PtNPs-S_1 bioconjugates were synthesized as follows: the peptide P_1 was firstly pre-incubated with TCEP for 40 min to prevent disulfide formation between peptides. Subsequently, 50 μL P_1 (2.5 μM) and 200 μL S_1 (2.5 μM) were successively subjected to 1 mL PtNPs solution under gentle stirring at 4 °C for 12 h. Next, to block extra active sites of PtNPs, 50 μL bovine serum albumin (BSA, 1.0 *wt* %) was added and reacted for 40 min. The resulting solution was centrifuged, washed three times, and then redispersed in 1.0 mL PBS. For comparison, we also prepared the P_1-AuNPs-S_1 bioconjugates in a similar method and the AuNPs were synthesized according to ref. 25.

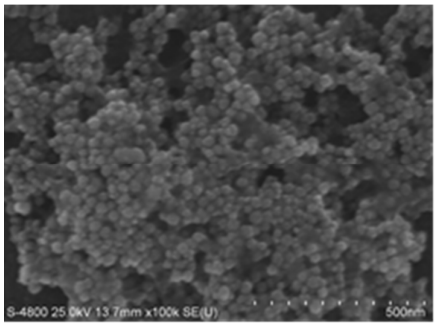
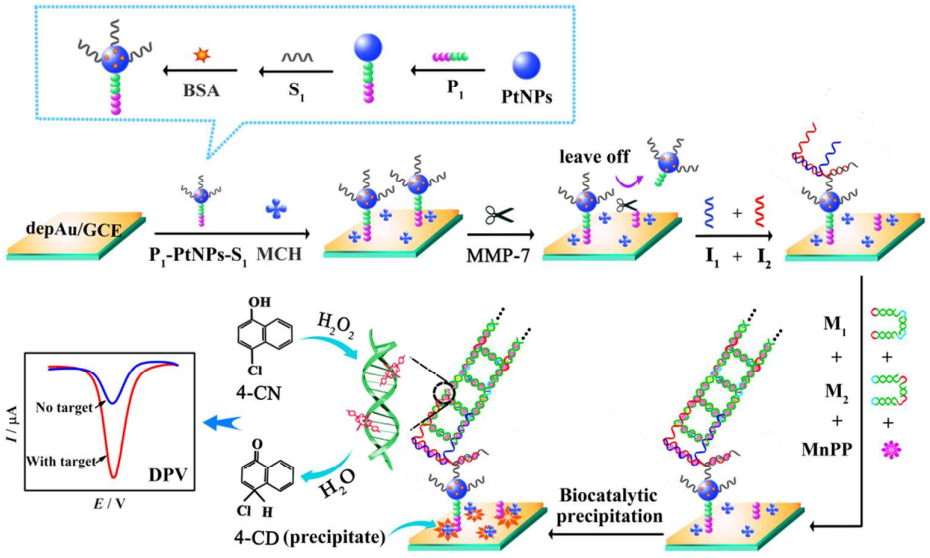


Fig. 1 SEM of the prepared PtNPs.

2.4 Assembly of the electrode

The cleaned GCE[26] was subjected to electrodeposition in 2 mL $HAuCl_4$ solution (1 *wt* %) (the potential: -0.2 V, deposition time: 30 s) to obtain Au nanoparticle layer

(depAu). 20 μL P_1-PtNPs-S_1 bioconjugates were then attached on the depAu modified electrode for 12 h at 4 °C, which followed by the incubation of 15 μL blocking agent MCH (2.0 mM). Next, 15 μL target MMP-7 was dropped onto the modified electrode with the incubation of 30 min at 37 °C. Then, the electrode was reacted with 20 μL mixture containing 4.0 μM I_1, 2.5 μM I_2, 0.5 μM motifs M_1, M_2 and 1.0 mM MnPP for 2 h, aiming to form DNA nanoladders for loading substantial MnPP. Finally, the modified electrode was dipped into 1.0 mM 4-CN containing 0.15 mM H_2O_2 and incubated for 15 min to induce enzymatic biocatalytic precipitation reaction.



Scheme 1 Schematic illustrations of electrochemical biosensor for matrix metalloproteinase-7 detection with DNA enzyme decorated DNA nanoladders as enhaner.

3. Results and discussions

3.1 Electrochemical characterization for different electrode

The cyclic voltammetry (CV) was utilized to study the interface properties of electrode. As depicted in Fig. 2A, bare GCE showed a well-defined redox peak (curve a). And the current increased after modification of conductive depAu (curve b). After the P_1-PtNPs-S_1 bioconjugates were assembled on electrode, the current slightly increased (curve c) for the conductive property of PtNPs. However, an obvious decline in peak current was detected after employing of MCH to block nonspecific binding sites (curve d). Once introduction of MMP-7, it could cause the cleavage of peptide and then release of PtNPs, resulting in a further decrease of current signal (curve e). As expected, when the electrode was treated with a mixture of I_1, I_2, M_1, M_2 and MnPP, the current response was reduced (curve f), this is because the formed DNA nanoladders with negative charges can repulse the positive charged redox probes. The peak current heavily decreased when the modified electrode incubated with the precipitation solution (curve g), which was mainly attributed to the fact that 4-CN was oxidized by biocatalysis of MnPP.

To further confirm that the generation of precipitation was induced by MnPP catalytic reaction, the proposed biosensor before and after the oxidization of 4-CN were compared by using DPV. As shown in Fig. 2B, before the oxidization of 4-CN, an obvious DPV peak was obtained (curve a). After MnPP participated the precipitation reaction of 4-CN, a very low current signal could be seen (curve b),

indicating that MnPP with excellent electrocatalytic performance toward 4-CN enhanced electrochemical signals.

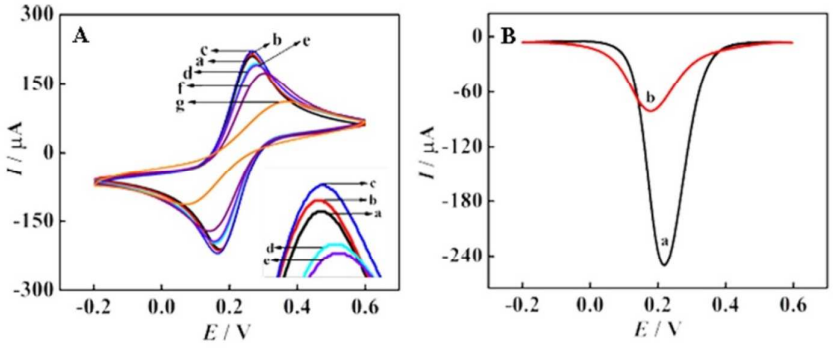


Fig. 2 (A) The CVs of the stepwise modified electrodes in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution: (a) bare GCE; (b) GCE/depAu; (c) GCE/depAu/P_1-PtNPs-S_1; (d) GCE/depAu/P_1-PtNPs-S_1/MCH; (e) the GCE/depAu/P_1-PtNPs-S_1/MCH after treated with MMP-7; (f) e + DNA nanoladders + MnPP; (g) f incubated in 1.0 mM 4-CN + 0.15 mM H_2O_2. (B) The DPVs for constructed biosensor (a) before and (b) after the precipitation assembly.

AFM was utilized to investigate the surface morphological of the process of biosensor fabrication. Fig. 3A showed an image of depAu on the electrode, which displayed spherical particles. Because peptide did not transmit electricity and hindered the electron transfer, we can observe that the films gradually became rough when the electrode was treated with P_1-PtNPs-S_1 bioconjugates (Fig. 3B). After incubating with MCH, MMP-7, mixture containing I_1, I_2, M_1, M_2 and MnPP in order, surface morphology of the modified films were changed obviously (Fig. 3C). This

was because target MMP-7 specifically recognized and cleaved the peptide, some of PtNPs were released from the electrode. When the modified electrode reacted with 1.0 mM 4-CN + 0.15 mM H_2O_2, more rough films could be observed (Fig. 3D), which was mainly attributed to the fact that MnPP was utilized as catalyst to electrocatalyze the 4-CN to generate indissoluble precipitation on electrode. These results also validated that the proposed electrochemical biosensor was successfully fabricated.

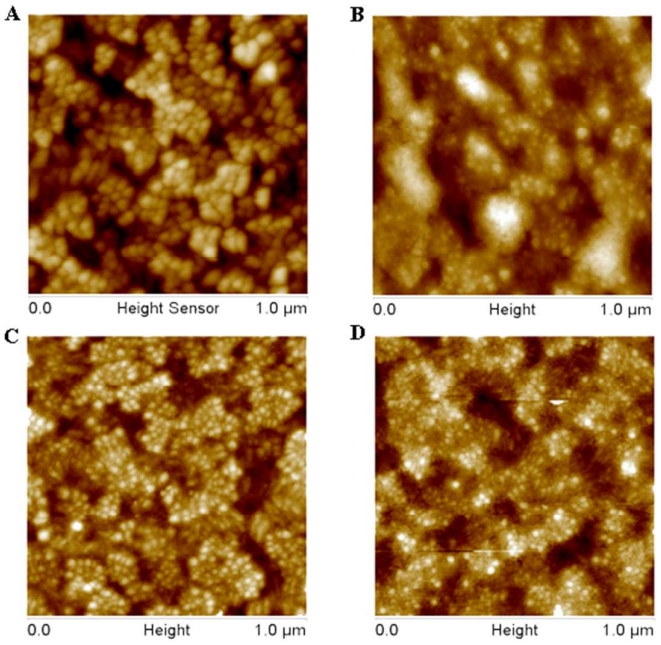


Fig. 3 AFM images of the modified films of: (A) depAu, (B) depAu/P_1-PtNPs-S_1, (C) the depAu/P_1-PtNPs-S_1/MCH after treated with MCH, MMP-7, DNA nanoladders and MnPP. (D) C incubated in 1.0 mM 4-CN + 0.15 mM H_2O_2.

At the same time, we also monitored the modified electrode surfaces with SEM. Fig. 4A displayed the electrodeposition of the gold nanoparticles on the bare GCE,

which presented a nanoflowerlike shape with the average size of 1 μm. However, the surface became fuzzy after the P_1-PtNPs-S_1 bioconjugate was modified on electrode (Fig. 4B), which suggested the successful modification of P_1-PtNPs-S_1 bioconjugate on electrode. After subsequent assembly with MCH, MMP-7, DNA nanoladders and MnPP, and indissoluble precipitation, the view became rough (Fig. 4C).

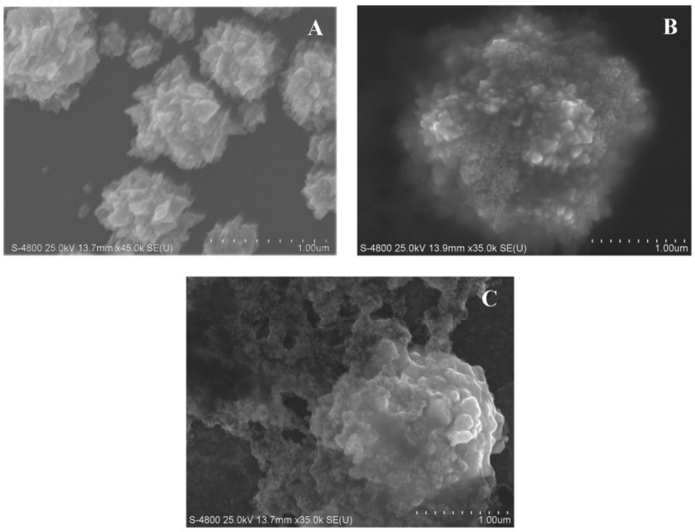


Fig. 4 The SEM of the modified films of (A) depAu, (B) depAu/P_1-PtNPs-S_1, (C) the depAu/P_1-PtNPs-S_1/MCH after treated with MMP-7, DNA nanoladders and MnPP, and then incubated in 1.0 mM 4-CN + 0.15 mM H_2O_2.

3.2 Comparison experiment

To test the signal amplification of MnPP associated nanoladders, the comparison experiment results of two different bioconjugates I_2-MnPP and DNA nanoladders-MnPP for biocatalytic precipitation were shown in Fig. 5. The change of current responses ($\Delta I = I - I_0$, where I_0 and I represented electrochemical signal

before and after amplification) was recorded to study the signal amplification effect with different methods. In Fig. 5A, peak current changed just about 88.68 μA when the biosensor was introducted with I_2-MnPP, while the biosensor with DNA nanoladders-MnPP, greatly increased to 132 μA (Fig. 5B). The reason was that the formed DNA nanoladders could increase the loading amount of MnPP to catalyze 4-CN to yield inert precipitation, suggesting the excellent signal amplification ability of MnPP associated DNA nanoladders.

PtNPs showed superior electrocatalytic efficiency toward reduction of H_2O_2, leading to an amplified electrochemical signal output. In order to elucidate the catalytic reduction of PtNPs toward H_2O_2, a comparative experiment was also designed in the same condition. As illustrated in Fig. 5C, biosensor incubated with P_1-PtNPs-S_1 bioconjugates (curve a) showed lower peak current than that of P_1-AuNPs-S_1 (curve b), demonstrating that PtNPs could efficiently catalyze the decomposition of H_2O_2, causing more inert precipitation on electrode.

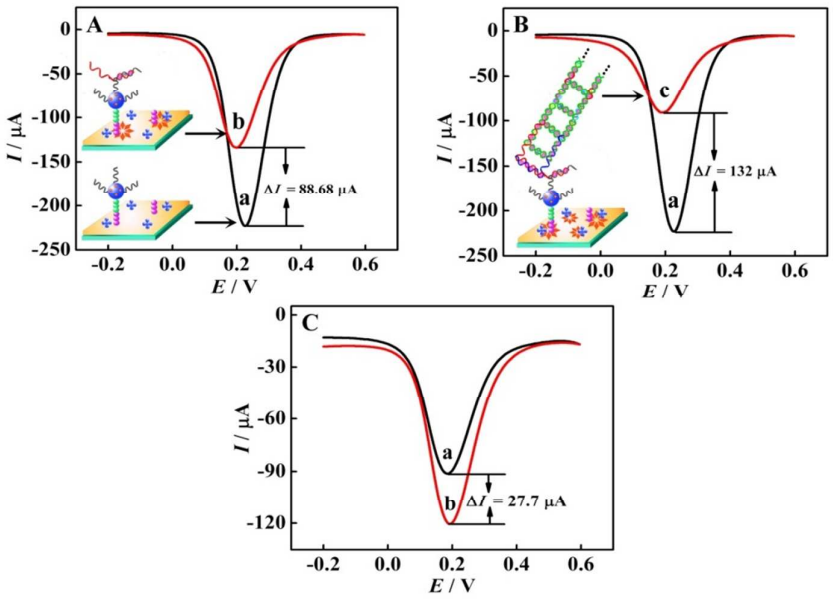


Fig. 5 The DPV responses of different strategies without (A) and with (B) DNA signal amplification: (a) GCE/depAu/P_1-PtNPs-S_1/MCH incubated with MMP-7; (b) a + I_2 +MnPP + 1.0 mM 4-CN + 0.15 mM H_2O_2; (c) a + DNA nanoladders + MnPP + 1.0 mM 4-CN + 0.15 mM H_2O_2. (C) The DPV signal of the developed biosensor incubated with (a) P_1-PtNPs-S_1 and (b) P_1-AuNPs-S_1.

3.3 Analytical performance of the biosensor for detection of MMP-7

Under the optimal condition (see Supporting Information, Fig. S2), the GCE/depAu/P_1-PtNPs-S_1/MCH was subjected to a series of concentrations of target MMP-7. As depicted in Fig 6, the DPV peak currents gradually increased (Fig 6A) and displayed a good liner relationship with increasing concentrations of MMP-7 varying from 0.2 pg/mL to 20 ng/mL (Fig 6B). The regression equation was I (μA) = -14.445 lg$c_{MMP\text{-}7}$ - 63.309 (r = -0.996) and the detection limit (LOD) was calculated 0.05 pg/mL. Additionally, the performance of proposed work was subjected to

comparison with that of some reported methods for MMP-7 detection. As shown in Table 1, our work showed higher sensitivity on account of DNA nanoladders and enzymatic biocatalytic precipitation for signal amplification.

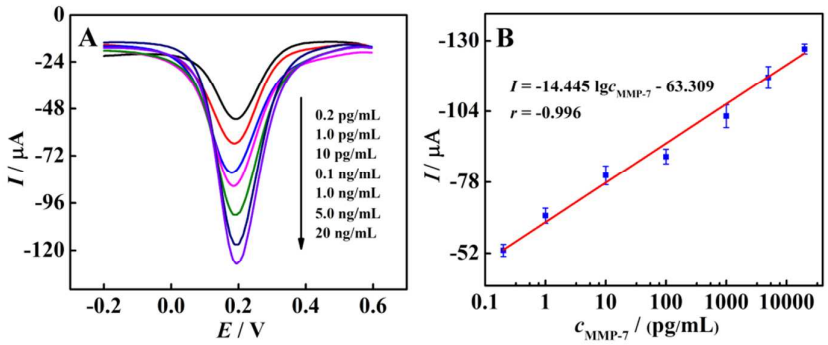


Fig. 6 (A) The DPVs of MMP-7 detection with different concentrations: 0.2 pg/mL, 1.0 pg/mL, 10 pg/mL, 0.1 ng/mL, 1.0 ng/mL, 5.0 ng/mL, 20 ng/mL; (B) The corresponding calibration curve.

Table 1 The performance of present work compared with some reported methods for MMP-7 detection.

Method for analysis	Detection limit	Linear range	Reference
colorimetric	0.1 μg/mL	0-2 μg/mL	7
SWV	3.4 pM	0-10 ng/mL	27
Scanometry	0.097 ng/mL	0.1-100 ng/mL	28
MS	----	20-2000 ng/mL	29
FETs	10 ng/mL	0.01-1 μg/mL	30
DPV	0.05 pg/mL	0.0002-20 ng/mL	Our work

Abbreviation: square wave voltammetry (SWV); mass spectrometry (MS); field-effect transistors (FETs); differential pulse voltammetry (DPV).

3.4 Selectivity, stability and reproducibility of proposed biosensor

To evaluate the selectivity of developed biosensor, several interferences such as hemoglobin (Hb), glucose and MMP-2 were tested. And the concentration with 10 times higher than that of MMP-7 was used for these interferences. As can be seen from Fig. 7A, the target MMP-7 induced a remarkable signal compared to that of other potential interferences. Additionally, the electrode was also incubated with a mixture of above four interferences containing MMP-7. Although these interferences are coexisted, the detection signal of the mixture had no apparent difference from MMP-7 only. These results indicated that the proposed biosensor exhibited superior specificity for MMP-7 detection.

We also investigated the reproducibility of proposed biosensor (Fig. 7B), and DPV responses were recorded from four electrodes at the same conditions. The electrochemical signal showed approximate value with the RSD of 5.68%. Additionally, a successive CV scans for 50 cycles was carried out after treated with 1 ng/mL MMP-7 (Fig. 7C), and the current decreased about 5.1% of its initial current, demonstrating an acceptable stability for proposed electrode.

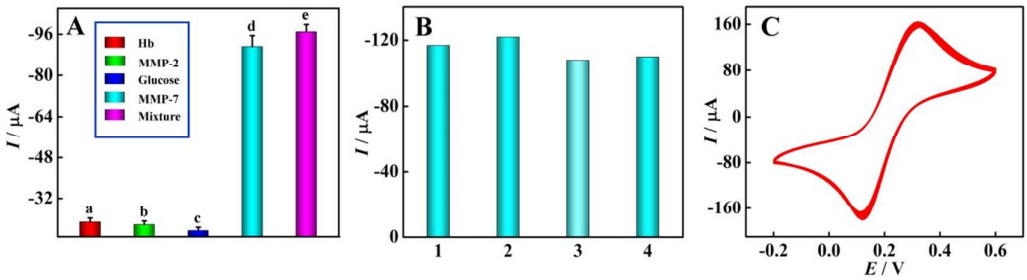


Fig. 7 (A) Specificity of electrode toward (a) 10 ng/mL Hb, (b) 10 ng/mL MMP-2, (c) 10 ng/mL glucose (d) 1 ng/mL MMP-7, and (e) 10 ng/mL Hb + 10 ng/mL MMP-2 + 10 ng/mL glucose + 1 ng/mL MMP-7. (B) Reproducibility assays of four biosensors prepared in the same conditions. (C) Stability of the proposed electrode under successive CV scans for 50 cycles.

4. Conclusion

In a word, a label-free, sensitive biosensor was successfully developed for detection of MMP-7 with DNA nanoladders and enzymatic biocatalytic precipitation for signal amplification. The remarkable signal amplification relied on the following points: (1) the DNA nanoladders with multiple DNA nanowires were served as ideal nanocarriers for loading numerous peroxidase mimic enzyme; (2) MnPP exhibited superior bioelectrocatalytic activities and catalyzed oxidization the 4-CN into insoluble precipitation in the presence of H_2O_2, amplifying the electrochemical signal; (3) the strategy avoided the fussy labeling procedure. In view of these excellent properties, our electrochemical analytical method can be extended easily for the detection of other proteases.

ASSOCIATED CONTENT

Supporting Information Characterization of DNA nanoladders; Optimization of the experimental conditions; Practical application of the biosensor.

ACKNOWLEDGMENT

This work was supported by the NNSF of China (51473136, 21575116, 21505107), the NNSF of CQ CSTC (cstc2016jcyjA0189), the Fundamental Research Funds for the Central Universities (SWU115037, XDJK2014C138) and Chongqing Graduate Research Innovation Project Foundation (CYS2015057).

REFERENCES

[1] Guan, P. P.; Yu, X.; Guo, J. J.; Wang, Y.; Wang, T.; Li, J. Y.; Konstantopoulos, K.; Wang, Z. Y.; Wang, P. By Activating Matrix Metalloproteinase-7, Shear Stress Promotes Chondrosarcoma Cell Motility, Invasion and Lung Colonization. *Oncotarget* **2015**, *6*, 9140-9159.

[2] Soum, C.; Rubio-Albenque, S.; Fery-Forgues, S.; Deleris, G.; Alouini, M. A.; Berthelot. T. Supramolecular Peptide/Surface Assembly for Monitoring Proteinase Activity and Cancer Diagnosis. *ACS Appl. Mater. Interfaces* **2015**, *7*, 16967-16975.

[3] Chiao, Y. A.; Zamilpa, R.; Lopez, E. F.; Dai, Q. X.; Escobar, G. P.; Hakala, K.; Weintraub, S. T.; Lindsey, M. L. In Vivo Matrix Metalloproteinase-7 Substrates Identified in the Left Ventricle Post-Myocardial Infarction Using Proteomics. *J. Proteome Res.* **2010**, *9*, 2649-2657.

[4] Prior, S. H.; Fulcher, Y. G.; Koppisetti, R. K.; Jurkevich, A.; Van Doren, S. R. Charge-Triggered Membrane Insertion of Matrix Metalloproteinase-7, Supporter of Innate Immunity and Tumors. *Structure* **2015**, *23*, 2099-2110.

[5] Fischbach, M.; Resch-Genger, U.; Seitz, O. Protease Probes that Enable Excimer Signaling upon Scission. *Angew Chem. Int. Ed.* **2014**, *53*, 11955-11959.

[6] Wang, X.; Xia, Y. Q.; Liu, Y. Y.; Qi, W. X.; Sun, Q. Q.; Zhao, Q.; Tang, B. Dual-Luminophore-Labeled Gold Nanoparticles with Completely Resolved Emission for the Simultaneous Imaging of MMP-2 and MMP-7 in Living Cells under Single Wavelength Excitation. *Chem. Eur. J.* **2012**, *18*, 7189-7195.

[7] Chen, P.; Selegard, R.; Aili, D.; Liedberg, B. Peptide Functionalized Gold Nanoparticles for Colorimetric Detection of Matrilysin (MMP-7) Activity. *Nanoscale* **2013**, *5*, 8973-8976.

[8] Xia, Z. Y.; Xing, Y.; So, M. K.; Koh, A. L.; Sinclair, R.; Rao, J. H. Multiplex Detection of Protease Activity with Quantum Dot Nanosensors Prepared by Intein-Mediated Specific Bioconjugation. *Anal. Chem*. **2008**, *80*, 8649-8655.

[9] Xie, S. B.; Ye, J. W.; Yuan, Y. L.; Chai, Y. Q.; Yuan, R. A Multifunctional Hemin@Metal-Organic Framework and Its Application to Construct an Electrochemical Aptasensor for Thrombin Detection. *Nanoscale* **2015**, *7*, 18232-18238.

[10] Dalui, A.; Pradhan, B.; Thupakula, U.; Khan, A. H.; Kumar, G. S.; Ghosh, T.; Satpati, B.; Acharya, S. Insight into the Mechanism Revealing the Peroxidase Mimetic Catalytic Activity of Quaternary CuZnFeS Nanocrystals: Colorimetric Biosensing of Hydrogen Peroxide and Glucose. *Nanoscale* **2015**, *7*, 9062-9074.

[11] Li, J. S.; Jia, Y. H.; Zheng, J.; Zhong, W. W.; Shen, G. L.; Yang, R. H.; Tan, W. H. Aptamer Degradation Inhibition Combined with DNAzyme Cascade-Based

Signal Amplification for Colorimetric Detection of Proteins. *Chem. Commun*. **2013**, *49*, 6137-6139.

[12] Wang, Q. Q.; Zhang, L. L.; Shang, C. S.; Zhang, Z. Q.; Dong, S. J. Triple-Enzyme Mimetic Activity of Nickel-Palladium Hollow Nanoparticles and Their Application in Colorimetric Biosensing of Glucose. *Chem. Commun.* **2016**, *52*, 5410-5413.

[13] Zheng, Y. N.; Yuan, Y. L.; Chai,Y. Q.; Yuan, R. L-cysteine Induced Manganese Porphyrin Electrocatalytic Amplification with 3D DNA-Au@Pt Nanoparticles as Nanocarriers for Sensitive Electrochemical Aptasensor. *Biosens. Bioelectron.* **2016**, *79*, 86-91.

[14] Zhou, X. X.; Xue, S. Y.; Jing, P.; Xu, W. J. A Sensitive Impedimetric Platform Biosensing Protein: Insoluble Precipitates Based on the Biocatalysis of Manganese (III) Meso-Tetrakis (4-N-Methylpyridiniumyl)-Porphyrinin in HCR-Assisted dsDNA. *Biosens. Bioelectron*. **2016**, *86*, 656-663.

[15] Guo, Y. N.; Wang, Y.; Liu, S.; Yu, J. H.; Wang, H. Z.; Wang, Y. L.; Huang, J. D. Label-Free and Highly Sensitive Electrochemical Detection of E. *coli* Based on Rolling Circle Amplifications Coupled Peroxidase-Mimicking DNAzyme Amplification. *Biosens. Bioelectron.* **2016***, 75,* 315-319.

[16] Zhu, Y.; Wang, H. J.; Wang, L.; Zhu, J.; Jiang, W. Cascade Signal Amplification Based on Copper Nanoparticle Reported Rolling Circle Amplification for Ultrasensitive Electrochemical Detection of the Prostate Cancer Biomarker. *ACS Appl. Mater. Interfaces* **2016**, *8*, 2573-2581.

[17] Bi, S.; Yue, S. Z.; Wu, Q.; Ye, J. Y. Triggered and Catalyzed Self-Assembly of Hyperbranched DNA Structures for Logic Operations and Homogeneous CRET Biosensing of MicroRNA. *Chem. Commun.* **2016**, *52*, 5455-5458.

[18] Xu, H. F.; Zhu, X.; Ye, H. Z.; Yu, L. S.; Chen, G. N.; Chi, Y. W.; Liu, X. X. A Bio-Inspired Sensor Coupled with a Bio-Bar Code and Hybridization Chain Reaction for Hg^{2+} Assay. *Chem. Commun.* **2015**, *51*, 15031-15034.

[19] Shen, W.; Deng, H. M.; Gao, Z. Q. Gold Nanoparticle-Enabled Real-Time Ligation Chain Reaction for Ultrasensitive Detection of DNA. *J. Am. Chem. Soc.* **2012**, *134*, 14678-14681.

[20] Yang, W. C.; Li, X. H.; Sun, J. L.; Shao, Z. F. Enhanced PCR Amplification of GC-Rich DNA Templates by Gold Nanoparticles. *ACS Appl. Mater. Interfaces* **2013**, *5*, 11520-11524.

[21] Nie, Z.; Wang, P. F.; Tian, C.; Mao, C. D. Synchronization of Two Assembly Processes to Build Responsive DNA Nanostructures. *Angew. Chem. Int. Ed.* **2014**, *53*, 8402-8405.

[22] Xie, H.; Wang, Q.; Chai, Y. Q.; Yuan, Y. L.; Yuan, R. Enzyme-Assisted Cycling Amplification and DNA-Templated in-Situ Deposition of Silver Nanoparticles for the Sensitive Electrochemical Detection of Hg^{2+}. *Biosens. Bioelectron.* **2016**, *86*, 630-635.

[23] Cheng, F. F.; He, T. T.; Miao, H. T.; Shi, J. J.; Jiang, L. P.; Zhu, J. J. Electron Transfer Mediated Electrochemical Biosensor for MicroRNAs Detection Based on

Metal Ion Functionalized Titanium Phosphate Nanospheres at Attomole Level. *ACS Appl. Mater. Interfaces* **2015**, *7*, 2979-2985.

[24] Jing, P.; Yi, H. Y.; Xue, S. Y.; Yuan, R.; Xu, W. J. A 'Signal On-Off' Electrochemical Peptide Biosensor for Matrix Metalloproteinase 2 Based on Target Induced Cleavage of Peptide. *RSC Adv.* **2015**, *5*, 65725-65730.

[25] Rai, M.; Singh, S. K.; Singh, A. K.; Prasad, R.; Koch, B.; Mishra, K.; Rai, S. B. Enhanced Red Upconversion Emission, Magnetoluminescent Behavior, and Bioimaging Application of $NaSc_{0.75}Er_{0.02}Yb_{0.18}Gd_{0.05}F_4$@AuNPs Nanoparticles. *ACS Appl. Mater. Interfaces* **2015**, *7*, 15339-15350.

[26] Xie, S. B.; Dong, Y. W.; Yuan, Y. L.; Chai, Y. Q.; Yuan, R. Ultrasensitive Lipopolysaccharides Detection Based on Doxorubicin Conjugated N-(Aminobutyl)-N-(Ethylisoluminol) as Electrochemiluminescence Indicator and Self-Assembled Tetrahedron DNA Dendrimers as Nanocarriers. *Anal. Chem.* **2016**, *88*, 5218-5224.

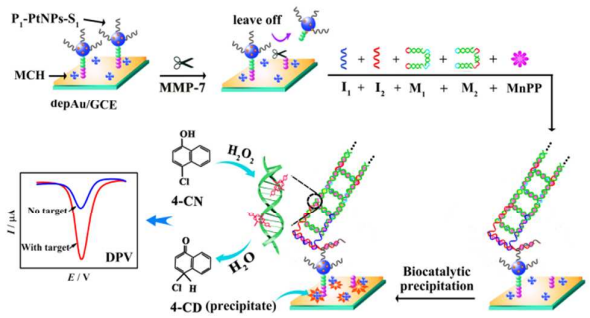
[27] Liu, G. D.; Wang, J.; Wunschel, D. S.; Lin, Y. H. Electrochemical Proteolytic Beacon for Detection of Matrix Metalloproteinase Activities. *J. Am. Chem. Soc.* **2006**, *128*, 12382-12383.

[28] Cheng, W.; Chen, Y. L.; Yan, F.; Ding, L.; Ding, S. J.; Ju, H. X.; Yin, Y. B. Ultrasensitive Scanometric Strategy for Detection of Matrix Metalloproteinases Using a Histidine Tagged Peptide-Au Nanoparticle Probe. *Chem. Commun.* **2011**, *47*, 2877-2879.

[29] Kim, Y. P.; Lee, B. S.; Kim, E.; Choi, I. S.; Moon, D. W.; Lee, T. G.; Kim, H. S. Activity-Based Assay of Matrix Metalloproteinase on Nonbiofouling Surfaces Using Time-of-Flight Secondary Ion Mass Spectrometry. *Anal. Chem.* **2008**, *80*, 5094-5102.

[30] Chen, H.; Chen, P.; Huang, J. F.; Selegard, R.; Platt, M.; Palaniappan, A.; Aili, D.; Tok, A. I. Y.; Liedberg, B. Detection of Matrilysin Activity Using Polypeptide Functionalized Reduced Graphene Oxide Field-Effect Transistor Sensor. *Anal. Chem.* **2016**, *88*, 2994-2998.

TOC


P1-PtNPs-S1
MCH
depAu/GCE
MMP-7
leave off
I1 + I2 + M1 + M2 + MnPP
4-CN
H2O2
H2O
4-CD (precipitate)
Biocatalytic precipitation
No target
With target
DPV
I / μA
E / V