Electronic Supplementary Information

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

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Experimental section

Chemicals and Materials

Carboxylated-magnetic polystyrene microspheres (PSC-COOH) was bought from Tianjin BaseLine ChromTech Research Centre (Tianjin, China). Exonuclease III (Exo III) and 10×NEBuffer 1 (1×NEBuffer 1, 10 mM bis-tris-propane-HCl, 1 mM dithiothreitol, 10 mM $MgCl_2$, pH 7.0 at 25 °C) were purchased from New England Biolabs (Beijing, China). Cucurbit[7]uril (CB[7]), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxy succinimide (NHS), tris (2-carboxyethyl) phosphine (TCEP) and gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were bought from Beijing Chemical Reagent Co. (Beijing, China). A specific peptide (NH_2-KKKPLGVRGCCC-SH) was synthesized and purified by Shanghai Science Peptide Biological Technology Co., Ltd (Shanghai, China). Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-7 (MMP-7) were obtained from Sino Biological Inc. (Beijing, China). All DNA oligonucleotides were

synthesized and purified by Sangon Biotech Co., Ltd (Shanghai, China). The nucleotide sequences were listed as follows:

DNA_1: 5'-AAAG ATA TCG CTG AGG TTG GTT GGAG-$(CH_2)_6$-SH-3'

MB-DNA_2: 5'-MB-AAC CAA CCT CAG CGA TAT-3'

Phosphate buffered solution (PBS, pH 7.0, 0.1 M) served as working buffer was prepared with 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. 20 mM Tris-HCl buffer (pH 7.4) containing 140 mM NaCl, 1 mM $CaCl_2$, 5 mM KCl and 1 mM $MgCl_2$ was used to prepare DNA oligonucleotide solution. All aqueous solutions were prepared with ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore). All other chemicals were of reagent grade and used as received.

In our experiment, the human serum samples were provided by Third Military Medical University. This study protocols had been approved by the local Institutional Review Board (IRB), and informed consent also was obtained from related patients. Sera were isolated by the centrifugation of whole blood at 3000 rpm for 20 min and subsequently kept frozen at -80 °C until analysis.

Apparatus

All electrochemical measurements including cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) experiments were conducted on a CHI 660E electrochemistry workstation (Shanghai Chenhua Instrument, Shanghai, China) with a conventional three electrode system including a platinum wire electrode as auxiliary electrode, a saturated calomel

electrode (SCE) as reference electrode and a bare or modified glassy carbon electrode (GCE) with 4 mm in diameter as working electrode. The pH measurements were performed with a pH meter (MP 230, Mettler Toledo, Switzerland).

Preparation of AuNPs-DNA$_1$ and bioconjugated magnetic nanoprobe

For preparing AuNPs-DNA$_1$, thiol-tethered DNA$_1$ was firstly activated by TCEP for 1 h at room temperature to reduce the disulfide bond. Then the solution was mixed with 16 nm AuNPs solution[1], and the mixture was incubated at 4°C for 16 h. After this step, salting treatments (0.5 M NaCl) were repeated three times, followed by an overnight incubation in dark to make the AuNPs-DNA$_1$ mature. And then, the solution was centrifuged at 12000 rpm for 20 min to remove excess reagents. Finally, precipitates were dispersed in Tris-HCl buffer and stored at 4 °C when not used.

The bioconjugated magnetic nanoprobe (PSC-peptide-AuNPs-DNA$_1$) was synthesized as follows. Firstly, 600 μL of PSC-COOH (5 mg·mL^{-1}) was activated by coupling reagents (200 mM EDC and 50 mM NHS) and then was mixed with 400 μL of peptide NH_2-KKKPLGVRGCCC-SH (10 μM) for 2 h to covalently couple peptide on the PSC-COOH surface *via* an acylation reaction. After being separated with magnetic separator and rinsed with 0.1 M PBS to remove uncoupled peptides, PSC-peptide was re-suspended in 1 mL of 0.1 M PBS solution. Subsequently, 1 mL prepared AuNPs-DNA$_1$ was added into above solution, and incubated on a rocking shaker for 12 h to assemble PSC-peptide to the surface of functionalized AuNPs (AuNPs-DNA$_1$) *via* Au-S bond. After another magnetic separation, the PSC-peptide-AuNPs-DNA$_1$ was re-suspended in 2 mL 0.1 M PBS solution containing 0.1% BSA

and incubated at 37 °C for 2 h to block the remaining active sites. Then the blocked PSC-peptide-AuNPs-DNA_1 was stored at 4 °C for the following experiment.

Exo III-assisted cycling amplification reaction for MMP-2 assay

The detailed procedure for Exo III-assisted cycling reaction was as follows. Firstly, 20 μL of prepared magnetic nanoprobe (PSC-peptide-AuNPs-DNA_1) was mixed with 20 μL of target MMP-2 in a plastic tube at 37 °C for 40 min to perform a cleavage reaction. After magnetic separation, the solution contained the AuNPs-DNA_1 was transferred into another tube to hybridize with 2 μM MB-DNA_2. After reaction for 60 min, 1 unit·μL^{-1} Exo III was added to the above solution and followed by incubating at 37 °C for another 60 min. Subsequently, the mixture was heated to 80 °C for 20 min to deactivate the Exo III. Finally, the obtained solution was cooled down to room temperature for further use.

Preparation of the functionalized electrode

Prior to use, the bare GCE was polished with 0.3 μm and 0.05 μm alumina powder respectively to obtain a mirror-like surface, and ultrasonically washed in ultrapure water and absolute ethanol, respectively. Then the GCE was electrodeposited in $HAuCl_4$ solution at -0.2 V for 30 s to obtain an Au nanoparticle layer (depAu). After that, the electrode was incubated with 1 mM CB[7] for 1 h at 37 °C, so that CB[7] could be firmly attached to depAu through the interaction between carbonyls and gold. After thoroughly cleaned with ultrapure water, the modified electrode was stored at 4 °C for further usage.

Experimental measurements

Before electrochemical measurements, the solution of Exo III-assisted cycling

reaction was dropped onto the surface of the CB[7]/depAu functionalized electrode for 1 h, and the electrode was thoroughly rinsed with ultrapure water. Then electrochemical measurements were performed on a CHI 660E electrochemistry workstation with a conventional three-electrode cell at room temperature. DPV measurements were carried out in 2 mL 0.1 M PBS buffer to investigate the performance of the biosensor. The parameters were as follows: potential scan range, -0.6~0.1 V; amplitude, 50 mV; pulse width, 0.05 s; pulse period, 0.2 s; sample width, 0.0167 s.

Results and discussion

Optimizations of the biosensor

The electrochemical response of the fabricated biosensor was depended on the amount of cleaved MB, which was affected by the incubation time of the Exo III. Thus, a time-course experiment of Exo III was conducted by using the 20 $ng \cdot mL^{-1}$ target MMP-2. As shown in Fig. S1A, with the increase of incubation time, the electrochemical signal increased and came to maximal value when the incubation time increased to 60 min. Thus, 60 min was considered to be the optimal incubation time of Exo III in the following experiments.

The MMP-2 cleavage time was another important parameter affecting the analytical performance of biosensor. Fig. S1B displayed the dependence of current intensity on incubation time of MMP-2. The current intensity increased with the increase of incubation time from 10 min to 40 min. When the incubation time was

prolonged from 40 min to 60 min, the current intensity nearly kept stable. Therefore, 40 min was adopted as the optimal cleavage time for MMP-2 in the following whole experiments.

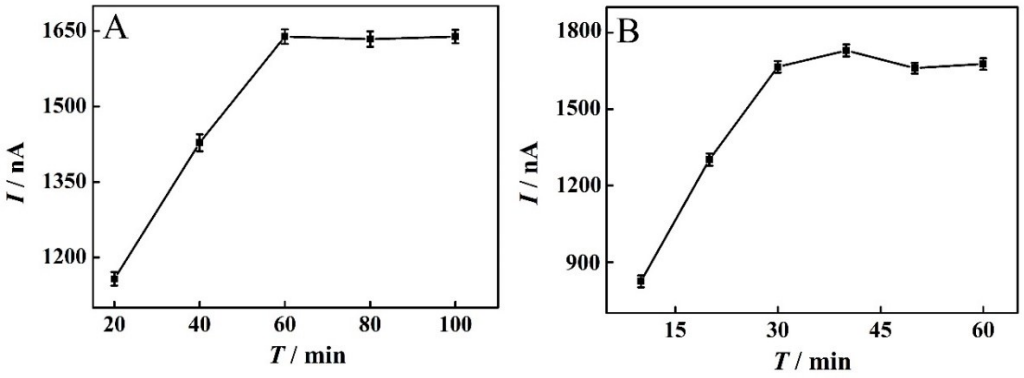

Fig. S1 The optimization of experimental parameters investigated by DPV experiments: (A) the effect of Exo III incubation time for enzyme-assisted cycling. (B) the incubation time of target MMP-2.

Table S1 Comparison of different methods for MMP-2 detection

Analytical method	Detection limit	Linear range	Ref.
FRET	12.5 ng·mL^{-1}	0.5-40 ng·mL^{-1}	2
FRET	2.5 ng·mL^{-1}	10-150 ng·mL^{-1}	3
BRET	2 ng·mL^{-1}	-----	4
Fluorescence	3.61 ng·mL^{-1}	14.4-144 ng·mL^{-1}	5
Potentiometry	2.6 ng·mL^{-1}	3.5-30 ng·mL^{-1}	6
DPV	0.15 pg·mL^{-1}	0.5 pg·mL^{-1}-50 ng·mL^{-1}	Our work

Abbreviation: SPR: surface plasmon resonance, FRET: fluorescence resonance energy transfer, BRET: bioluminescent resonance energy transfer, DPV: differential pulse voltammetry.

Table S2 Determination of MMP-2 added in human serums (n = 3) with the developed biosensor

Sample number	Added/(ng·mL^{-1})	Found/(ng·mL^{-1})	Recovery/%	RSD/%
1	0.010	1.005×10^{-2}	100.50	5.34
2	0.10	9.406×10^{-2}	94.06	2.31
3	0.50	0.5116	102.32	4.32
4	1.0	0.9655	96.55	3.65

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