Supporting information

Electrochemical Proteolytic Beacon for Detection of Matrix Metalloproteinase

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Experimental

Materials All peptide synthesis chemicals were analytical reagents or better and purchased from Applied Biosystems, Inc.(Foster City, CA) or Fisher. Ferrocene acetate acid, sodium perchloride, Brij® 35 solution(30%, w/v) and Tricine(>98%) were bought from Sigma-Aldrich. MMP-2, MMP-3 and MMP-7 were obtained from EMD biosciences Inc.(San diego, CA). Chemicals and solvents were purchased and used without further purification. Solutions were prepared with ultrapure water from a Millipore Milli-Q water purification system (Billerica, MA, USA).

Safety Considerations. Piranha (H_2SO_4/H_2O_2=7/3) and 1:1 HNO_3 solutions used in the procedures for cleaning the gold and glass surface are highly corrosive and should be handled in a fumehood. Skin and eye contact and accidental inhalation or ingestion should be avoided.

Peptide synthesis

The peptide with a sequence R-P-L-A-L-W-R-S-C was synthesized by a conventional stepwise solid-phase peptide synthesis method using the Fmoc protection. The synthetic

peptide was purified by C_{18} reverse-phase HPLC. The molecular weight of the purified peptide is 1328.3, which was verified by mass spectrometry analysis (ESI-MS method, see Figure S1). A larger m/z peak at 665 (m/z (2+)) represents the FC-peptide in a form of multiply charged ions (MH^{2+}). The larger m/z peaks (between 1328 to 2000) reflect various larger molecular species. Several of the peaks can be accounted for as Fe adducts (such as 1383.7 [1+] and 692.7 [2+]). Other masses are likely to represent oxidation products resulting from the preparation. The similar kind of distribution in the 685-1329 m/z range is also observed, these likely represent the doubly protonated ions (m/z at 665) of these same series of molecular species.

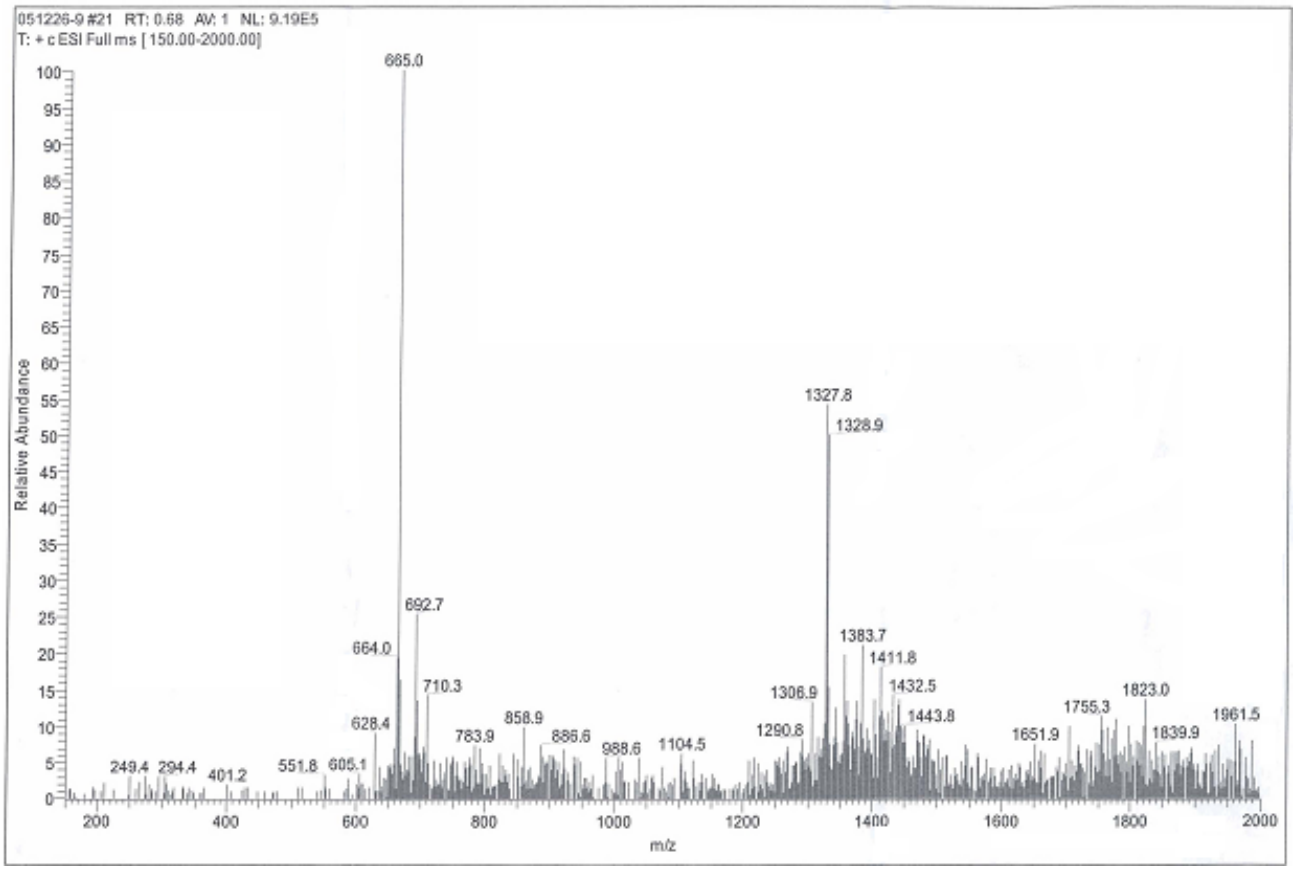

Figure S1 Mass-Spectrum of Ferrocene-R-P-L-A-L-W-R-S-C conjugate

Preparation of Ferrocene acetate acid-peptide conjugate

The method of conjugating the FC is the same as that of conjugating regular amino acids. After synthesis of the peptide, the peptide resin was treated with ferrocene acetic acid in TBTU, HoBt and DIPEA in a reaction vessel. The molar ratio of the ferrocene actate acid, TBTU, HoBt, DIPEA, and the peptide resin is 3: 3.3 : 3.3 : 3.3 : 1. The conjugation reaction lasted for about 3 h at room temperature. Then, the crude conjugated peptide was cleaved from the resin and purified, in a manner similar to that for regular peptides.

Preparation of FC-peptide – Au electrode

The FC-peptide conjugate possesses a thiol group at the C terminal for immobilization on gold substrate by self-assembling method.[1] Prior to the experiment, gold electrode (2 mm diameter, CH instrument) was polished carefully to a mirror-like surface with 0.3 and 0.05μm alumina slurry, respectively, and sonicated 10 min in 1:1 HNO3, acetone and water, respectively. The cleaned gold electrode was scanned cyclically within the potential range 0.5 to 1.5V in freshly prepared 0.2 M H_2SO_4 until voltammogram characteristic of the clean polycrystalline gold was established. The gold electrode was washed with distilled water and dried in a stream of dry nitrogen gas. The self-assembling of FC-peptide on the gold surface was performed 24 hrs at 4 ^{0}C by dipping the electrode into an ethanol solution containing 10 μM FC-peptide conjugate.[S1] The FC-peptide-Au electrode was thoroughly rinsed with ethanol, backfilled with 1 mM mercaptohexanol and used for electrochemical experiment.

Fourier Transform Infrared Reflection-Absorption spectroscopy (FTIR-RAS)

The molecular orientation of the FC-peptide SAMs was studied by Fourier transfer infrared reflection-absorption spectroscopy (FTIR-RAS). FTIR-RAS spectra were recorded on a Bruker IFS66v/S Fourier Transform Infrared Spectrometer. For RAS measurements, a Harrick model RMA-1DG/VRA reflection attachment was used. The incident angle was set at 85^0. Gold substrates for Fourier FTIR-RAS measurements were prepared by vapor deposition of chromium (50 nm) as an adhesion layer and then gold (99.99%, 200 nm) on slide glass. The slide glasses were cleaned ahead of deposition with a piranha solution (H_2SO_4/H_2O_2=7/3), followed by rigorous rinsing with distilled water and methanol. Spectra were collected at 4 cm^{-1} resolution over the frequency range of 5200 to 500 cm^{-1}, with 16 cm^{-1} phase resolution, with a zero filling factor of 2, using a Blackman Harris three term apodization, and Mertz phase correction. A 16 kHz low pass filter was used to prevent aliasing. The tilt angle of the helix axis was 34^0 based on the amide I/amide II absorbance (Figure S2), indicating that the helix axis in each SAM was oriented. [2]

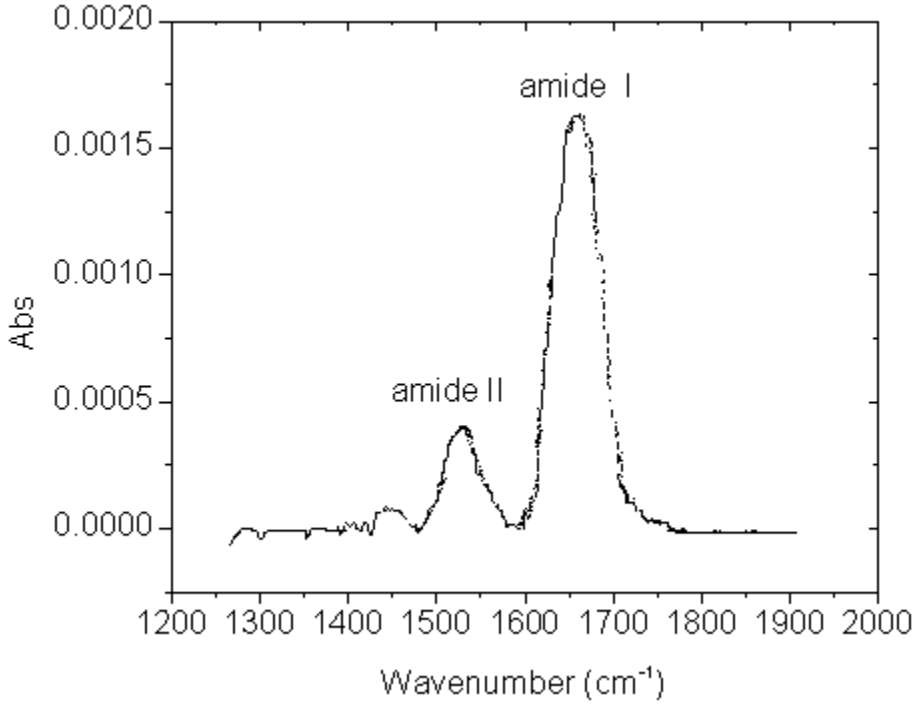

Figure S2 FTIR-RAS spectra in the amide region of Fc-peptide self-assembling monolayer on gold substrate

Batch electrochemical measurements

Square-wave voltammetric and cyclic voltammetric measurements were performed with an electrochemical analyzer CHI 621A (CH Instruments, Austin, TX) connected to a personal computer. A three-electrode configuration was employed, consisting of a FC-peptide-Au electrode (2 mm diameter) serving as a working electrode, while Ag/AgCl/(3 M KCl) and platinum wire served as the reference and counter electrode, respectively. For the batch conditions, cyclic voltammetric and square voltammetric measurements were performed in 2–mL electrochemical cell containing 0.6 M $NaClO_4$ supporting electrolyte.

Electrochemical characteristics of the FC-peptide-Au electrode

The electrochemical characteristics of ferrocene acetate acid and FC-peptide-Au gold were studied with cyclic voltammetry under batch conditions. Figure S3 presents the

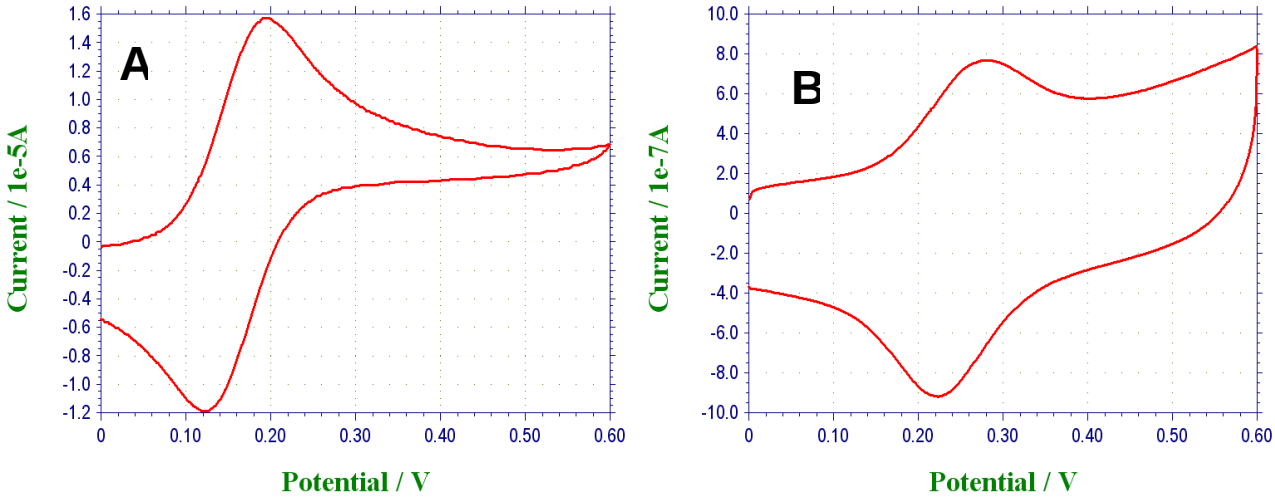

Figure S3 (A) Cyclic voltammogram of ferrocene acetate acid in 0.6 M $NaClO_4$ solution, working electrode: gold electrode; **(B)** Cyclic voltammogram of FC-peptide-Au electrode in 0.6 M $NaClO_4$ solution. Scanning rate: 100 mv/s.

cyclic voltammograms of standard ferrocene acetate acid solution with gold working electrode (A) and FC-peptide-Au electrode (B) in 0.6 M $NaClO_4$ solution. We can see the redox potentials (Epa, 0.26 V, Epc, 0.21 V, vs Ag/AgCl) of FC in FC-peptide conjugate shift around 100 mV comparing with that of free FC (Epa,0.19 V, Epc, 0.12V) , which represents a slow electron transfer of helix peptide due to the long-range electron transfer reaction.

Figure S4 (A) presents the cyclic voltammograms of FC-peptide-Au electrode with different scanning rate. The redox peak currents were increasing with the increase of potential scanning rate and directly proportional to scan rates, implying electrochemical

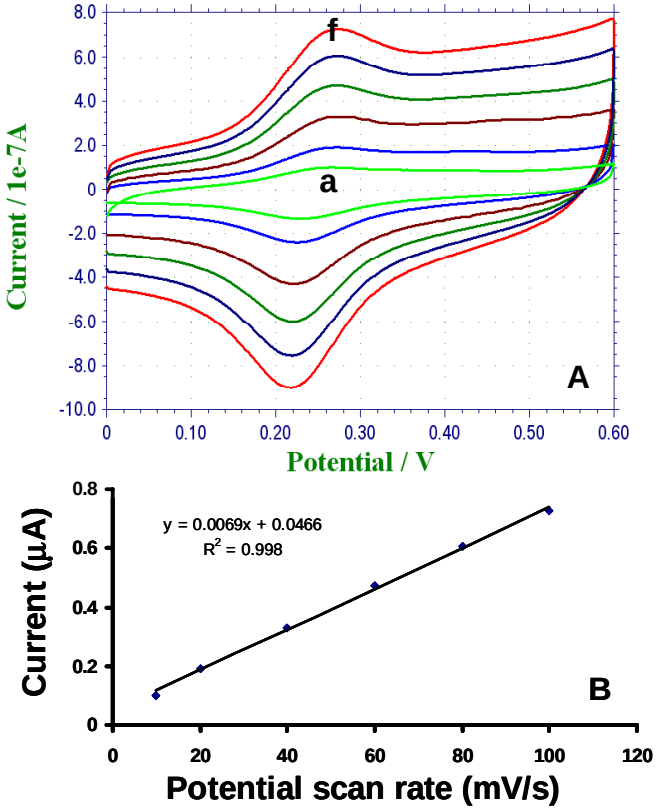

Figure S4 (A) Cyclic voltammograms of FC-peptide-Au electrode at different potential scanning rate, from a to f, 10, 20, 40, 60, 80, 100 mv/s, respectively; (B) The relationship plot of oxidation peak current of FCA& scan rate.

reactions of surface-bound redox species.[3] The surface coverage (Γ) of FC-peptide was estimated from the area of the cyclic voltammetric peaks corresponding to the oxidation of ferrocene centers. According to the equation $\Gamma = Q/nFA$,[3] where Q is the area of the FC oxidation peak, n is the moles of electrons involved in the FC oxidation peak(n=1), F is the Faraday's constant, and A is the area of the electrode(A=0.0314 cm^2). Γ was found to be 0.103 $nmoL/cm^2$ in our studying.

Stability studies of FC-peptide-Au electrode

Signal stability of the FC-peptide-Au electrode was also tested by cyclic voltammetry. **Figure S5** shows the voltammogram of 40 successive potential scanning cycles with the FC-peptide-Au electrode in 0.6 M NaClO4 solution. The arrows in Figure S5 indicate the

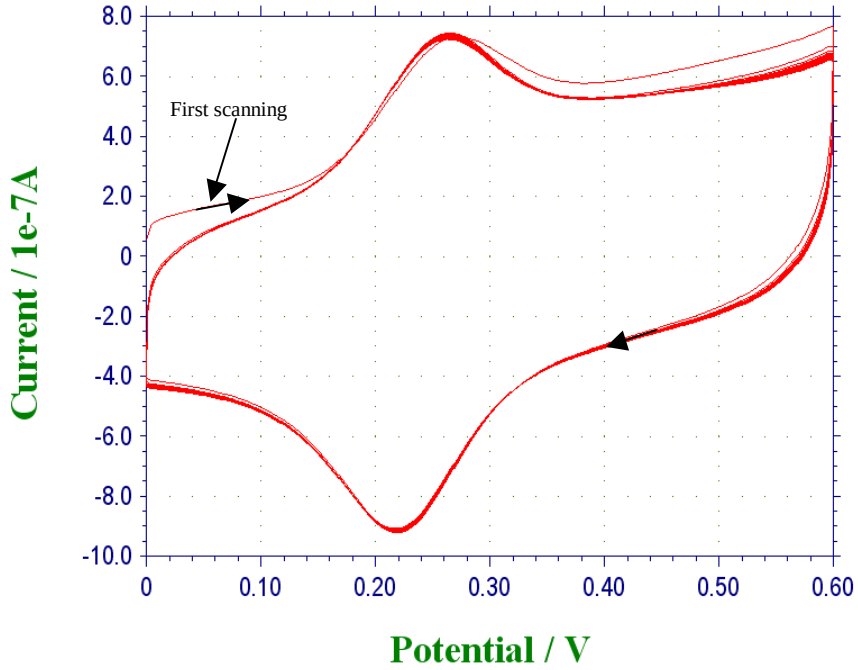

Figure S5 Cyclic voltammogram of FC-peptide-Au electrode in 0.6 M $NaClO_4$ solution. Scanning cycles: 40; scanning rate: 100 mV/s.

potential scanning direction. One can see the FC-peptide-Au electrode exhibits good stability. Similar stability was also observed with a pretreated FC-peptide-Au electrode, which was incubated 30 min with 5 ng mL^{-1} MMP-7 (results not shown).

Effect of $NaClO_4$ concentration on the signal of FC-peptide-Au electrode

The concentration of $NaClO_4$ has significant effect on the signal of FC-peptide-Au electrode. Figure S6 (A) presents the square voltammograms of FC-peptide-Au electrode in different concentrations of $NaClO_4$. One can see the SWV signal of FC is increasing

with the increase of $NaClO_4$ concentration, then saturates at 0.6 M. Also shown the relationship between the peak current and the concentration of $NaClO_4$ (Figure S6(B).

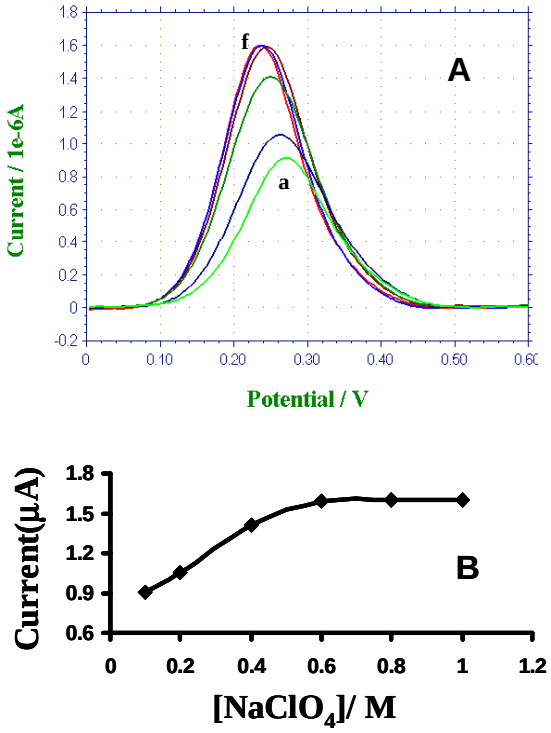

Figure S6 (A) Square voltammograms of FC-peptide-Au electrode in different concentrations of $NaClO_4$, from a to f, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 M, respectively. (B) The relationship plot of FC peak current & $NaClO_4$ concentration.

Flow injection/electrochemical measurement of MMP-7 acitivity

In the flow injection system, three electrodes were inserted into a home-made wall-jet flow cell. The cell volume is about 40 μl. The $NaClO_4$ supporting electrolyte was delivered using a syringe pump (Model 362, ORION Research Inc.) at a flow rate of 0.25 ml/min. The sample solution was injected with a low-pressure 6-Port injection valve (model V 540, UPCHURCH Scientific). The typical flow injection procedure for the MMP-7 activity measurement is following:

1. Deliver 0.6 M $NaClO_4$ at 250 μl /min to flow cell, then stop flow and follow a square wave voltammetric measurement.
2. Inject 40 μL of Tricine buffer containing desired concentration of MMP-7 into the cell, and stop flow 30 min for cleavage.
3. Deliver 1mL of 0.6 M $NaClO_4$ to the cell and follow a square wave voltammetric measurement.

During the time course studies (the relationship between cleavage time and the remaining SWV signal (percentage) of the FC-peptide--Au electrodes), the cleavage time was controlled by the stop flow time. Each measurement at specific cleavage time was performed with a freshly prepared FC-peptide-Au electrode. For each set of the tests, three FC-peptide-Au electrodes were used.

References:

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2. Miura, Y.; Kimura, S.; Imanishi, Y.; Umemura, J. *Langmuir* **1998**, 14, 6935.

3. Bard, A.J.; Faulkner, L. R. Electrochemical Methods, Wiley, New York, **2001**.