Supporting Information

Disposable Capillary-fill Device for the Determination of Proteases Incorporating Eliminating Light-shield of Magnetic Beads with Cleavage of the Electrogenerated Chemiluminescence Label-tagged Peptide Probe

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Table of Content

1.1 Regents and apparatus

The conductive carbon paste (LOCTITE EDAG-423SS) was purchased from Loctite China Co., Ltd (China). The conductive silver and silver chloride past (Ag/AgCl=8/2，CIE-4250) was purchased from Guang Zhou City Silver Well trading Co., Ltd (China). PET substrates (3.5 cm × 2.5 cm, thickness=0.3 mm) and glass plate (3.3 cm × 2.5 cm, thickness=1.2 mm) were purchased from Xi'an Beiyin Biotechnology Co., Ltd (China). The small magnet (φ=8.0 mm thickness=2.0 mm) was obtained from Shenzhen Min Magnetic Technology Co., Ltd. (China).

UV‑Vis spectra were recorded with a UV-2450 UV-vis spectrophotometer (Shimadzu, Japan). Fluorescence spectra were performed on a Cary Eclipse Fluorescence Spectrophotometer (Agilent). An Olympus IX-51 inverted microscope (Olympus Corporation, Tokyo, Japan) that was equipped with a mercury lamp (Olympus) and Olympus Dual CCD DP80 camera were used to obtain fluorescence images, in which a filter set from Chroma with excitation filter centered at 490 nm (BP470-490), emission filter centered at 630 nm (BA520IF) which corresponds to the ruthenium complex. The transmission electron microscope (TEM) was performed on a FEI Tecnai G2 F20 Field Transmitance Electron (Oregon, USA).

1.2 Synthesis of the MB ECL probes

The MBs ECL probes (MBs@SA•biotin-peptide-Ru1) were synthesized by binding biotinylated specific peptide with streptavidin-coated magnetic beads and then covalently coupling Ru1 with the lysine (K) of the peptide via an acylation reaction.[1] The chemical structure of the Ru1 and biotin-HSSKLQK peptide were present in Fig. S1. Firstly, 0.70 mg of the biotinylated specific peptide (biotin-HSSKLQK, 0.67 μmol) was dissolved in 1.0 mL of 1×PBS and then mixed with 750 μL of streptavidin-coated magnetic beads (10 mg/mL, φ=2.8 μm), incubated on a rocking shaker for 4 h. After being separated with magnetic separator and repeated washing with 1× PBS to remove uncoupled peptides, the MBs-peptide was re-suspended in 750 μL of 1×PBS. Secondly, 100 μL of DMF containing 1.0 mg $Ru(bpy)_2(mcbpy\text{-}O\text{-}Su\text{-}ester)(PF_6)_2$ was added into above suspension, incubated on a rocking shaker for 12 h. After being separated with magnetic separator and rinsed with 1× PBS to remove uncoupled Ru1. The MBs@SA•biotin-peptide-Ru1 were re-suspended in 100 μL of 0.1% BSA, incubated at 37 ℃ for 2 h to block any remaining active surfaces. The blocked MBs@SA•biotin -peptide-Ru1 were rinsed for three times with 1× PBS and re-suspended in a final volume of 750 μL of 1× PBS as the MBs ECL probe and stored at 2-8 °C. The concentration of MBs ECL probe was 10 mg /mL according to the amount of MBs@SA in case of no loss of MBs@SA. The work of the MBs ECL probe was diluted using 1× PBS to different concentrations as needed.

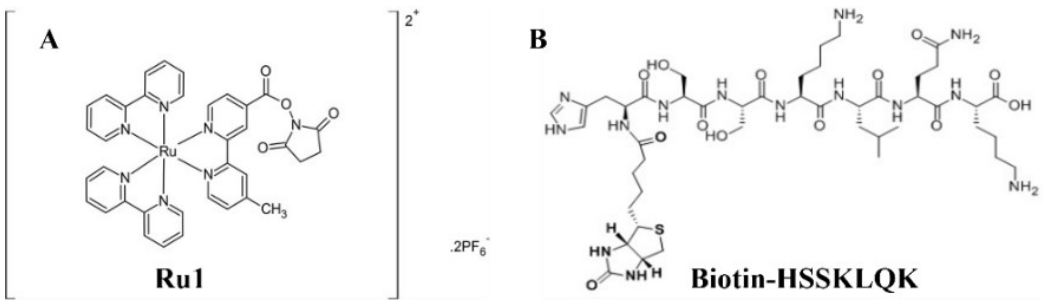

Fig. S1 Chemical structure of the Ru1 (A) and biotin-HSSKLQK peptide (B).

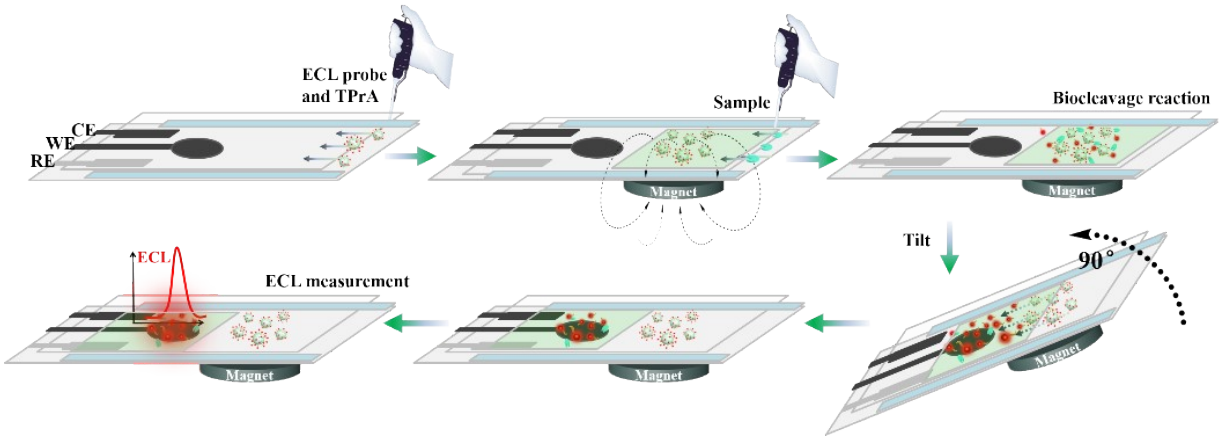

Fig. S2 Schematic representation of the ECL bioanalytic system for the determination of the PSA.

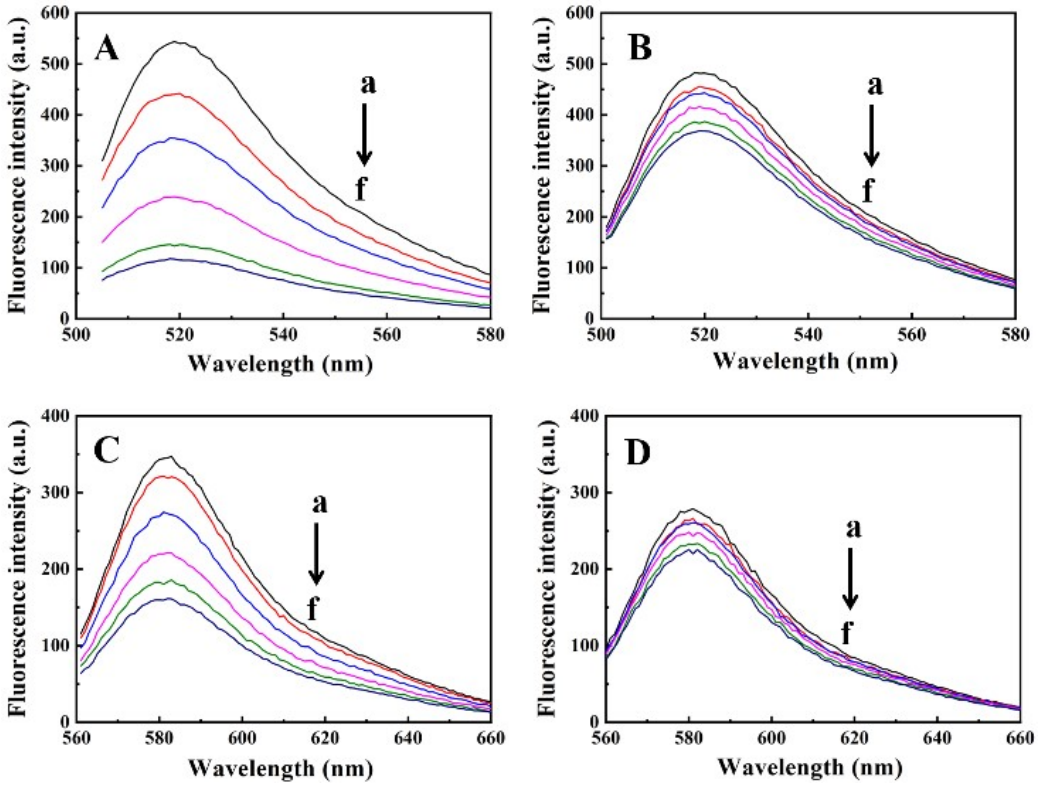

Fig. S3 Fluorescence spectra of 2.0 μM FITC solution mixed with different concentrations of 0.20 μm MBs (A) and 2.2 μm MBs (B), λ_{ex}=493 nm, split width =5 nm; The fluorescence spectra of 5.0 μM TRITC solution mixed with different concentrations of 0.20 μm MBs (C) and 2.2 μm MBs (D), λ_{ex}=550 nm, split width =5 nm. a-0, b-0.020, c-0.05, d-0.10, e-0.15 and f-0.20 mg/mL.

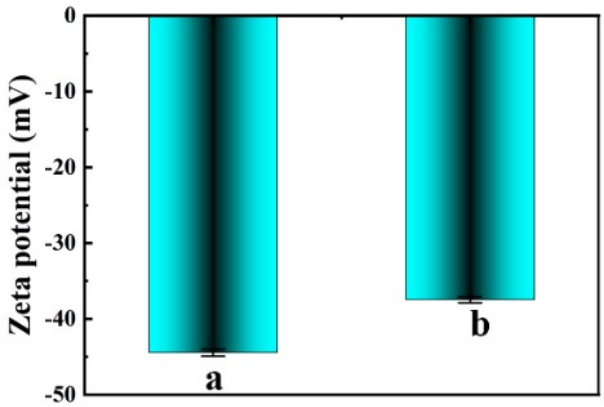

Fig. S4 Zeta potential of the MBs@SA (a) and MBs@SA•Peptide-Ru1 (b)

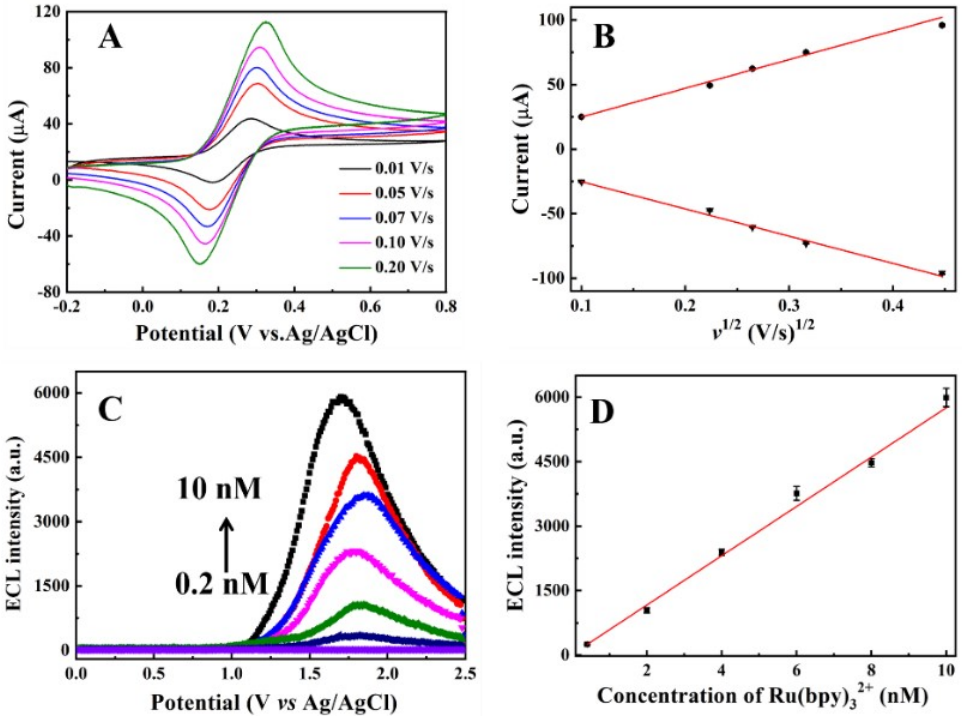

Fig. S5 (A) Cyclic voltammograms at the prepared SPCE (φ= 6.0 mm) in 0.10 M PBS (pH 7.40) containing1.0 mM Fc-CH_2OH with different scan rates (0.01-0.20 mV/s); (B) Dependence of the peak currents on the square root of scan rates at SPCE. (C) The ECL intensity-potential curves at SPCE (φ= 6.0 mm) in 0.10 PBS (pH 7.40) containing 30 mM TPrA and different concentration of $Ru(bpy)_3Cl_2$ (0.2 to 10 nM) with a scan rate of 100 mV/s; PMT=-900V. (D) Dependence of the peak ECL intensity on the concentration of $Ru(bpy)_3Cl_2$.

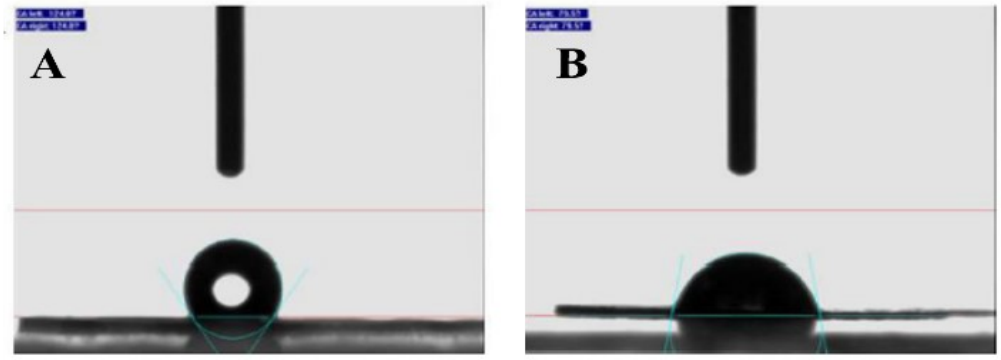

Fig. S6 Contact angle of the SPCE before (a) and after (B) preoxided treatment.

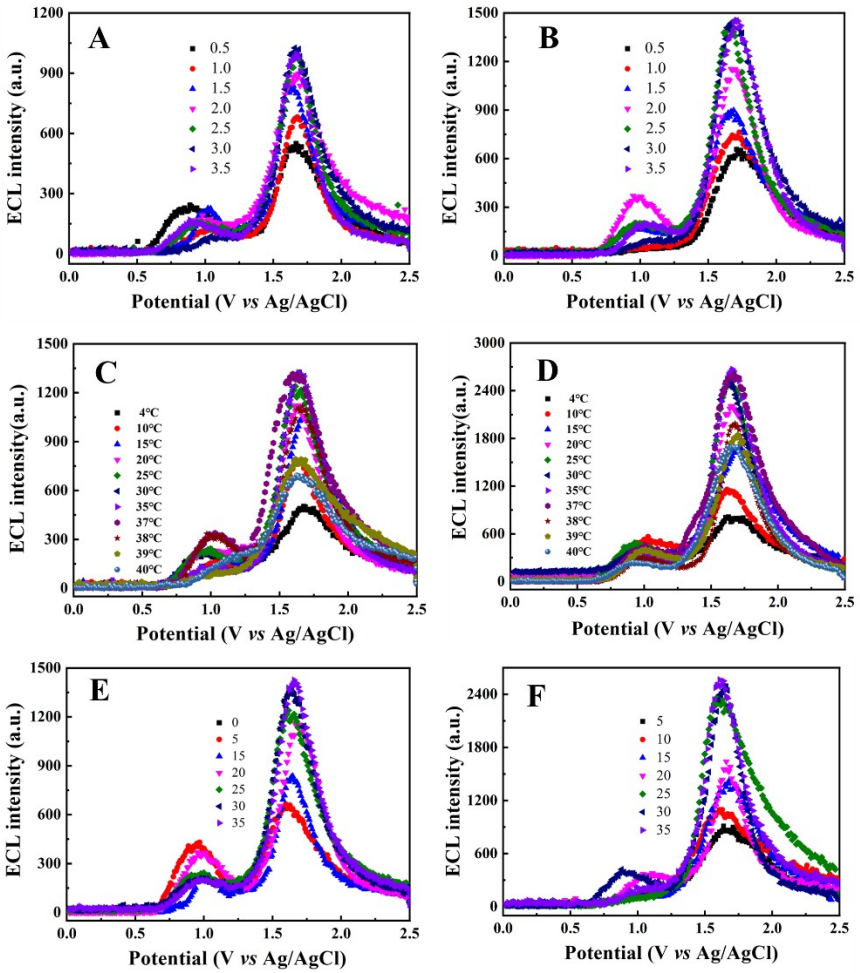

Fig. S7 The ECL intensity-potential profiles of different concentration of MBs ECL probe after adding (A) 0.6 ng/mL and (B) 1.2 ng/mL PSA at the disposable ECL capillary-fill device. The ECL intensity-potential profiles at disposable ECL capillary-fill device containing 2.5 mg/mL MBs ECL probe after adding (C) 0.6 ng/mL and (D) 1.2 ng/mL PSA under different temperature. The ECL intensity-potential profiles at disposable ECL capillary-fill device containing 2.5 mg/mL of MBs ECL probe after adding (E) 0.6 ng/mL and (F) 1.2 ng/mL PSA at 30°C with different cleavage time. The ECL measurement was carried out in the 0.10 PBS (pH 7.40) containing 50 mM TPrA with a scan rate of 100 mV/s. PMT= -900 V.

Table S1 Comparison of the linear range and detection limit reported method with that of the developed method for the determination of PSA

Method	Linear range	Detection limit	Time (h)	Ref
Photothermal	1.0 to 50 ng/mL	0.40 ng/mL	2.0	[2]
Photothermal	1.0 to 64 ng/mL	2.1 ng/mL	4.0	[3]
Fluorescence	3.4 to 34 ng/mL	1.7 ng/mL	8.5	[4]
Fluorescence	4.4 to 71.3 ng/m	4.4 ng/mL	/	[5]
Fluorescence	0.2 to 12 ng/mL	0.06 ng/mL	0.25	[6]
Electrochemistry	2.0 to 10 ng/mL	1.95 ng/mL	1.0	[7]
Electrochemistry	1.0 to 200 ng/mL	0.40 ng/mL	1.25	[8]
Chemiluminescence	1.0 to 100 ng/mL	0.60 ng/mL	0.50	[9]
ECL	2.25 to 11.3 ng/mL	0.88 ng/mL	4.0	[10]
ECL	0.20 to 1.2 ng/mL	0.12 g/mL	0.50	This work

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