Supporting Information for;

An electrochemical biosensor for detection of the sepsis-related biomarker procalcitonin

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Chemicals

Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Human recombinant procalcitonin proteins were purchased from Randox Life Science (Crumlin, UK). A series of synthetic peptides modified with a C-terminal cysteine, and a flexible linker (-GGGGS-) were chemically synthesized (> 95% purity) by Peptron (Daejeon, Korea), as shown in Table S1. The quartz crystals deposited with gold were obtained from Biolin Scientific (Stockholm, Sweden). Phosphate-buffered saline (PBS, pH 7.4) and Tris-HCl buffer solutions were used to make all proteins, human plasma samples, and synthetic peptide solutions for CV and EIS measurements. Unless otherwise stated, all reagents were analytical grade.

Preparation of the affinity peptide-modified electrode

The peptide-functionalized gold electrode was prepared by the following steps. First, the gold electrode was immersed into piranha solution (H_2SO_4:H_2O_2 = 4:1, v/v) for 10 min to remove residual dust and impurities on the surface layer of the gold electrode. The electrode was then rinsed with deionized (DI) water several times. This pre-functionalized electrode was dried by blowing with N_2 gas for several seconds. After these polishing steps, the electrode and voltammetric cell were assembled, and 100 μL of thiol-modified synthetic peptides (50 μg/mL) were dropped onto the gold electrode and incubated at room temperature for 1 h. To remove unbound synthetic peptides, the cell was washed with 1X PBS (pH 7.4) and then again with DI water. Next, 2 μL of pure procalcitonin proteins was loaded onto each assembled cell and then incubated at room temperature for 1 h. The cells were again sequentially washed with 1X PBS and DI water to remove the unbound proteins.

CV and EIS measurements

A conventional three-electrode system, including a working electrode, platinum counter electrode, and Ag/AgCl reference electrode, was used for the electrochemical measurements. CV and EIS were performed using an electrochemical analyzer (CHI 750E, CH Instruments, Austin, TX, USA) connected to a computer data analysis system. These analyses were conducted in a PBS solution with 4 mM ferro/ferricyanide. The CV was recorded from 0.0 V to +0.4 V vs. an Ag/AgCl electrode at a sweep rate of 20 mV/s. EIS was carried out at a formal DC potential of 0.2 V using an alternating voltage of 10 mV in the frequency range from 10 Hz to 10 kHz.

Table S1. The affinity synthetic peptides used in this study

Names	Sequence	Predicted pI*[1]	Predicted M.W*[2]	Notes
PCT BP1	MSCAGHMCTRFVGGGGC	8.06	1673.99	Selected upon biopanning Used as peptide scaffold for rational design
PCT BP2	MSCAGDMCTEFVGGGGC	3.55	1624.87	Substitution of His to Asp at position of 6 Substitution of Arg to Glu at position of 10 Used to determine the effect of polar (positive) amino acid residues on binding interactions
PCT BP3	TSCNGHTCTRYNGGGGC	8.06	1687.80	Substitution of Met to Thr at position of 1 and 7 Substitution of Ala to Asn at position of 4 Substitution of Phe to Tyr at position of 11 Substitution of Val to Asn at position of 12 Used to determine the effect of neutral amino acid residues on binding interactions
PCT BP4	MSGAGHMGTRFVGGGGC	8.55	1581.81	Substitution of Cys to Gly at positions of 3 and 8 Used to determine the effect of cysteine amino acid on binding interactions

*[1] and *[2] : The prediction of isoelectric point (pI) and molecular weight of the affinity peptides were performed at Peptide Property Calculator (http://www.genscript.com).

Table S2. Analytical performance for the detection of procalcitonin

Detection method	Target	Limit of detection (LOD)	Reference
Immunoluminometric assay	procalcitonin	0.1 ng/mL	[1]
Sandwich-type electrochemical immunosensor	procalcitonin	0.5 pg/mL	[2]
Optical immunosensor	procalcitonin	88 μg/mL	[3]
Sandwich immunoassay	procalcitonin	2.5-2.6 μg/mL	[4]
Affinity peptide sensor with electrochemical detection	procalcitonin	12.5 ng/mL	**This study**

Additional references;

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Figure S1. The Principle of operation of our sensor used in this study. A) Schematic illustration of basic principle of our sensor system. B) Equivalent circuit model for EIS measurements.

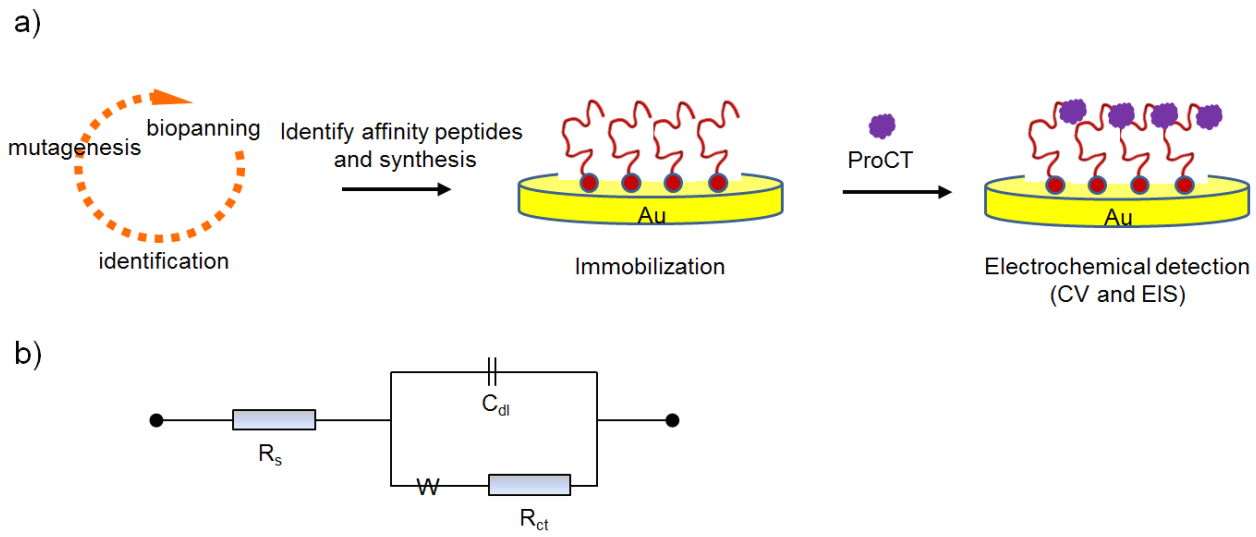

Figure S2. The performance of the sensor. a) Stability test of the sensor. PCT BP3 peptide (2.5 mg/mL) was immobilized on the gold electrode with different time range (0-5 hr) at room temperature and added ProCT or BSA. The R_{ct} value was measured by EIS. b) Effect of serum on binding interactions. Binding affinity of the PCT BP3 was comparable in the presence of 0.5% FBS compared with ProCT alone. In addition, PCT BP3 peptides do not have sufficient binding affinity to BSA as a negative control.