Supplementary Information for

Design of Bi-functional Peptide for Protein Assay: Observation of Cortactin Expression in Human Placenta

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Experimental Section

Reagents and Chemicals.

The bi-functional peptide and its C-terminal truncated version (11-mercaptoundecanoic acid (MUA)-GLSKKRPPPPPPGHKRT-CONH-KHG-NH_2, and MUA-GLSKKRPPPPPPG, lyophilized powder, purity>95%.) were custom-synthesized by Chinaoligopeptide Co, Ltd. Recombinant human cortactin (>98%) was purchased from ProSpec-Tany TechnoGene Ltd. Thermolysin, 9-mercapto-1-nonanol and MUA were purchased from Sigma-Aldrich. Other reagents were all of analytical-grade. The stock solution of the peptide was prepared by dissolving the powder as a 5 μM solution with 10 mM phosphate buffer (pH 7.4). The standard sample of cortactin was prepared by dissolving the powder with 10 mM PBS (pH 7.4) and then diluting it to desired concentrations with the same buffer. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of 18 MΩ·cm. Placenta microvilli samples from pregnant women of different trimester was obtained from Department of Gynecology and Obstetrics, the First Affiliated Hospital of Nanjing Medical University after elective cesarean, which were approved by the local hospital ethical committees. Pretreatment of the fresh microvilli sample was conducted using a Nuclear Extract Kit (Active Motif, CA). The tissue sample was diced and homogenized, followed by fractionation, and then the cytoplasmic fraction of protein sample was collected for cortactin detection. All the quantification and comparison of cortactin level in tissue sample were normalized according to the weight of the placenta sample used.

Gold Electrode Treatment and Modification.

Firstly, gold disk electrode (3 mm diameter) was cleaned using piranha solution (70% concentrated sulfuric acid, 30% H_2O_2) for 5 min (*Caution: Piranha solution reacts violently with organic solvents and should be handled with great care!*). After that, the electrode was rinsed with double-distilled water. The electrode was then polished with 1 μm, 0.3 μm alumina slurry in sequence. Subsequently, ultrasonicating in both ethanol and water was used to remove residual alumina powder. Finally, the electrode was immersed in nitric acid (50%) for 30 min, followed by electrochemically cleaning with 0.5 M H_2SO_4 to remove remaining impurities.

After being dried with nitrogen, the electrode was immerged in 50 μL assembly solution (2.5 μM bi-functional peptide and 5 mM TCEP in 10 mM PBS, pH 7.4) for 16 h at 4 °C, TCEP was used to prevent disulphide formation between peptides. Then, the electrode was soaked in 100 μL MNH solution (1 mM MNH in 10 mM PBS, pH 7.4) for 3 h at room temperature. Finally, MNH non-specifically adsorbed on the electrode surface was removed by thorough rinsing of the modified electrode, which was then dried under mild nitrogen stream.

Detection of Cortactin.

The modified electrode was firstly interacted with standard or biological samples containing cortactin for 2.5 h at room temperature. After that, the electrode was thoroughly rinsed with double-distilled water and was then dipped in 5% Tween-20 for 30 min to exclude non-specific adsorption. Then the target-bound electrode was subject to thermolysin cleavage (50 μl, 7nU/ml in 50 mM Tris-HCl, 0.5 mM $CaCl_2$ and trace amount of $ZnCl_2$, pH 8.0) for 2 h at 50 °C. After the cleavage was stopped by adding 1 μl 0.2 M EDTA, the electrode was thoroughly rinsed with double-distilled water and 5% Tween-20 once again. Subsequently, the electrode was incubated with 100 μM copper chloride solution for 1 h at room temperature. Signal response of the coordinated copper ion was then recorded. For signal amplification, the copper ion-labeled electrode was dipped in a 1 mM HCl solution (1 ml) containing 0.1 mg/ml OPD. The solution was placed in 50 °C water bath for 30 min. After cooled to room temperature, this solution was then buffered with 4ml 10 mM PBS pH 7.4. Signal response of the generated DPA was then recorded in the buffered reaction solution.

Electrochemical Measurements.

Electrochemical measurements were carried out at room temperature on a CHI660C Potentiostat (CH Instruments) with a conventional three-electrode system: the modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode. Cyclic voltammograms (CV) and square wave voltammograms (SWV) were recorded in 10 mM PBS, pH 7.4, which was deoxygenated by purging with nitrogen gas and maintained under this inert atmosphere during the above electrochemical scans. Experimental parameters for the response of coordinated copper ion are as follows. Cyclic voltammetry: scan range, -0.1 ~ 0.6 V, scan rate, 0.1 V/s. Experimental parameters for the amplified response of DAP were as follows. Cyclic voltammetry: scan range, -0.5 ~ -0.1 V, scan rate, 0.1 V/s. Square wave voltammetry: scan range, -0.5 ~ -0.1 V, step potential, 5 mV, frequency, 15 Hz, amplitude, 25 mV. The experimental parameters for EIS: bias potential, 0.224 V vs. SCE; amplitude, 5 mV; frequency range, 0.1 Hz ~ 10 kHz. electrolyte solution: 5mM $Fe(CN)_6^{3-/4-}$ with 1 M KCl. The data are obtained from at least three times of repetition of independent experiment, error bars are shown in the figures.

Signal response of DAP for the detection of cortactin

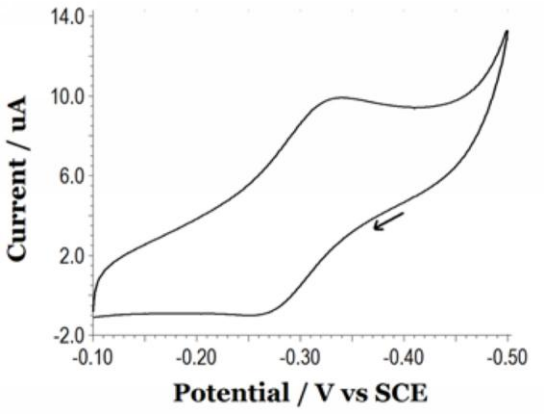

Figure S1. The cyclic voltammogram of DAP for the detection of cortactin. DAP is generated after copper ion-catalyzed OPD oxidation. This reaction is conducted by inserting the peptide-modified electrode into 1 ml substrate solution containing 1 mM HCl and 0.1 mg OPD for 30 min 50 °C water bath. Before this reaction, the peptide-modified gold electrode has been previously treated in sequence with 5.13 ng/ml cortactin, 7nU/ml thermolysin and 100 µM $CuCl_2$. Scan rate: 0.1 v/s. The arrow marks scan direction.

Optimization of incubation time of cortactin

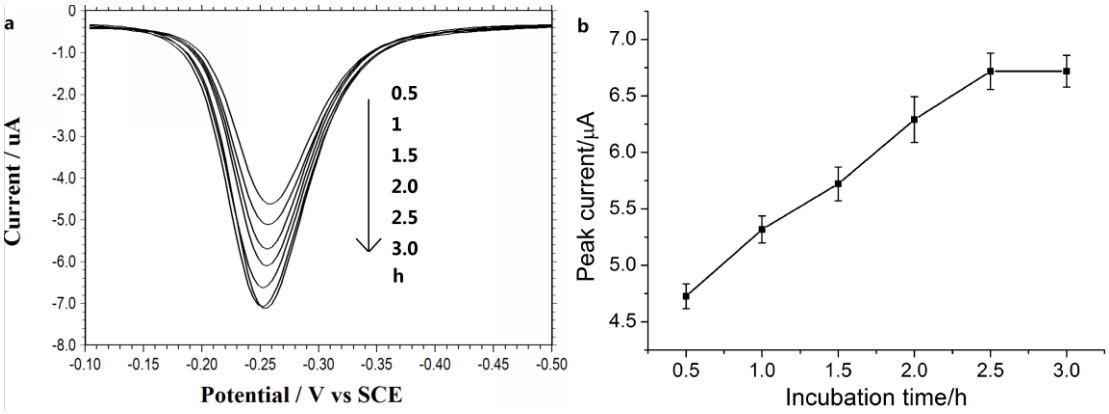

Figure S2. (a) Square wave voltammograms (SWVs) of DAP obtained after the same treatment as Figure S1, except that the peptide-modified electrode is incubated with cortactin for different time. The experimental parameters for square wave voltammetry: scan range, -0.5 ~ -0.1 V; step potential, 5 mV; frequency, 15 Hz; amplitude, 25 mV; electrolyte solution, PBS 10 mM pH 7.4. Panel (b) shows peak currents in (a) as a function of the optimized experimental parameter. The error bars represent standard deviation of the average (n=3).

Optimization of cleavage time of thermolysin

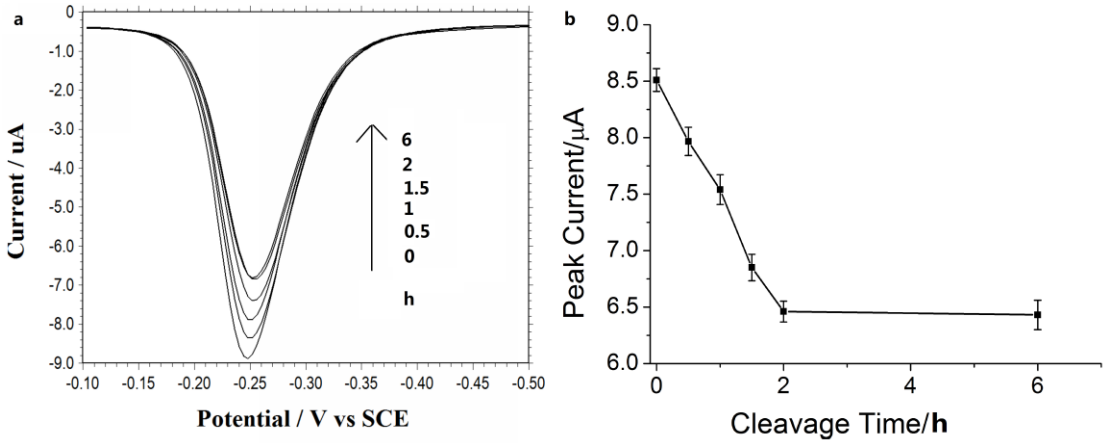

Figure S3. (a) SWVs of DAP obtained after the same treatment as Figure S2, except that the cortactin-bound electrode is incubated with thermolysin for different time. All the other conditions are the same as Figure 2a. The meaning of points and curves in panel b are the same as Figure S2b.

Optimization of concentration of copper ion

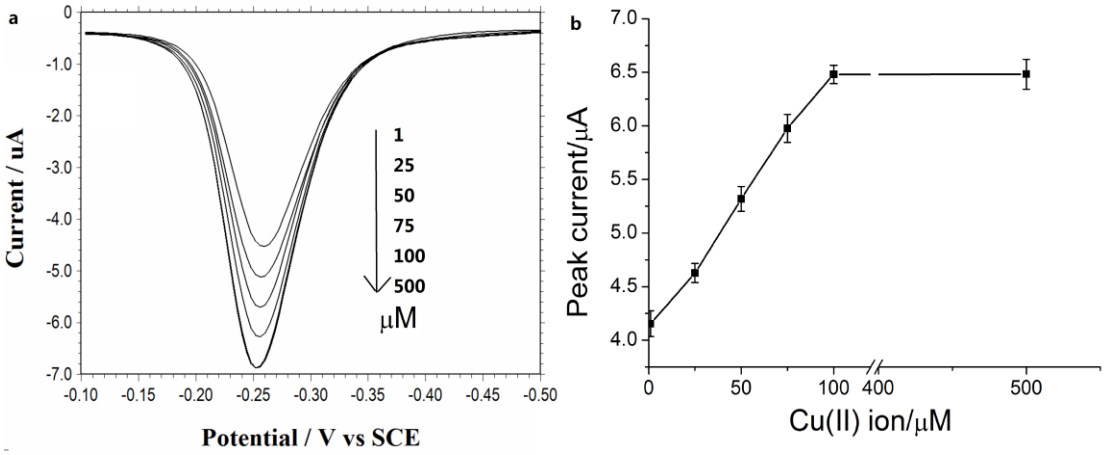

Figure S4. (a) SWVs of DAP obtained after the same treatment as Figure S3, except that the thermolysin-treated electrode is incubated with different concentration of $CuCl_2$. All the other conditions are the same as Figure S2a. The meaning of points and curves in panel b are the same as Figure S2b.

Optimization of concentration of OPD

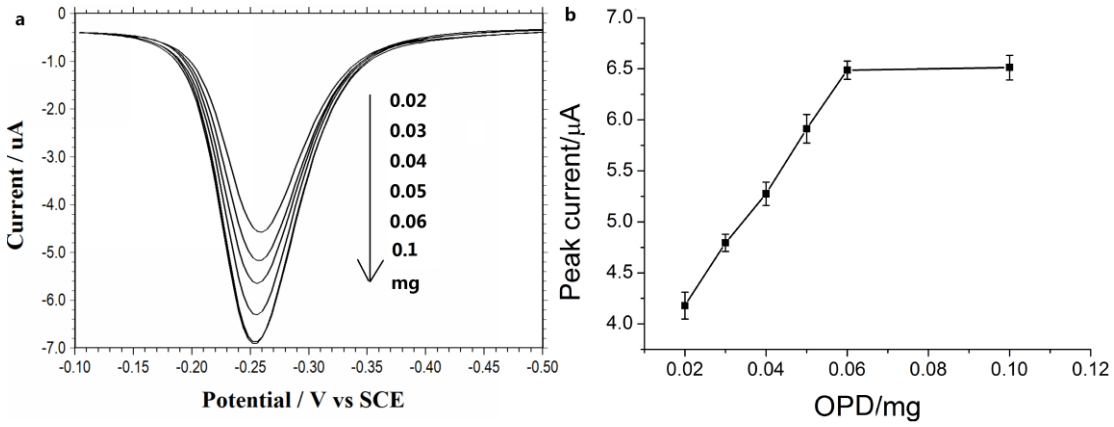

Figure S5. (a) SWVs of DAP obtained after the same treatment as Figure S3, except that the copper ion-coordinated electrode is incubated with different concentration of OPD. All the other conditions are the same as Figure S2a. The meaning of points and curves in panel b are the same as Figure S2b.

Detection of cortactin in placenta sample

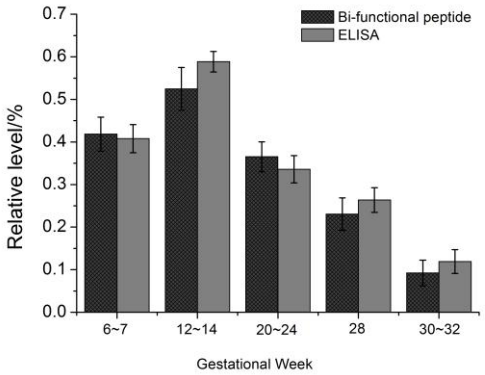

Figure S6. Detected cortactin level in placenta microvilli sample retrieved at different gestational weeks. The level is expressed as a percentage elevation relative to the cortactin level of placenta tissue sampled upon delivery. The error bars represent standard deviation from the average of three samples prepared from the same piece of retrieved placenta tissue.