Selective and Direct Immobilization of Cysteinyl Biomolecules by Electrochemical Cleavage of Azo Linkage

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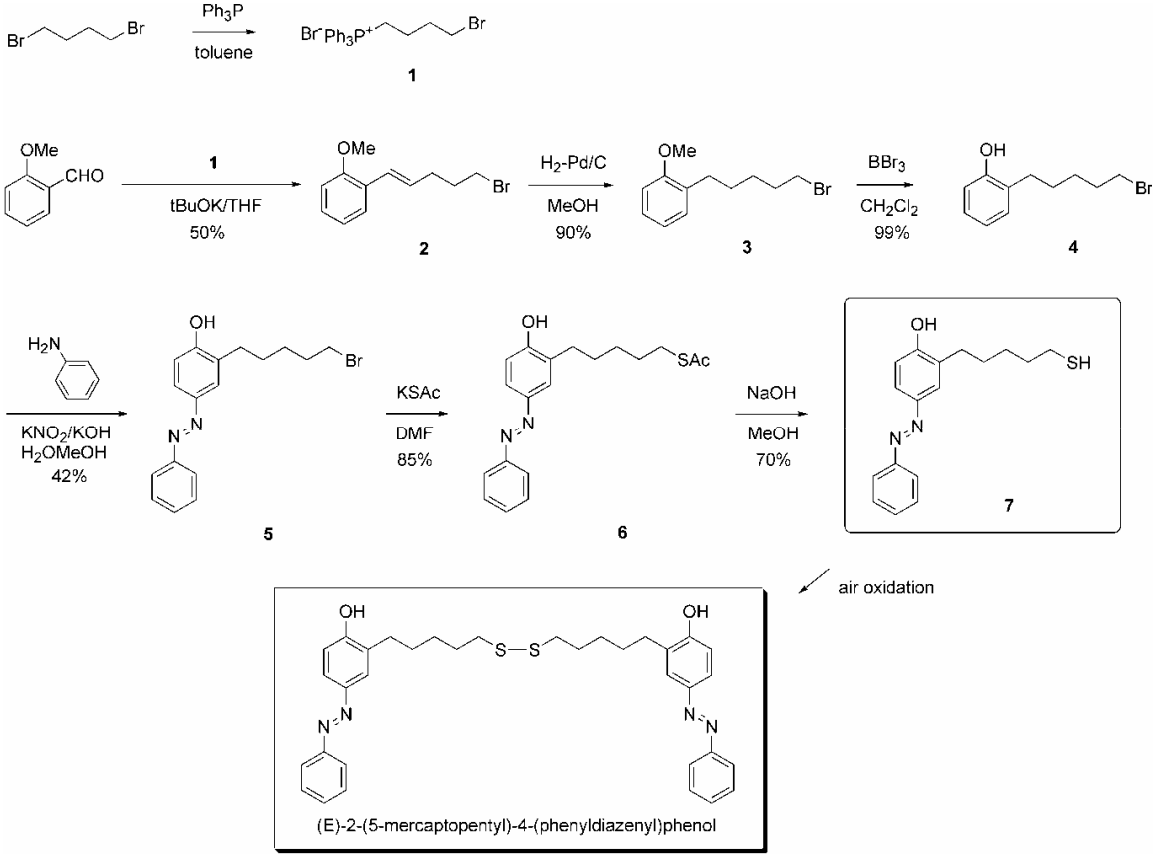
Supporting Information

Materials. Absolute ethanol was purchased from Merck (Whitehouse Station, NJ). Boric acid, acetic acid, L-cysteine, sodium tetraborate, tris(2-carboxyethyl)phosphine (TCEP), β-lactoglobulin, horse heart myoglobin were from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide was from J.T. Baker (Phillipsburg, NJ). Phosphoric acid, potassium chloride, potassium ferricyanide, sulfuric acid, nitric acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide were form Daejung (Seoul, Korea). (E)-2-(5-Mercapto-pentyl)-4-(phenyldiazenyl)-phenol was synthesized (Scheme S1) and provided by Medigen (Daejeon, Korea).

Scheme S1. Synthetic process of (E)-2-(5-Mercapto-pentyl)-4-(phenyldiazenyl)phenol.



Preparation of SAMs. Gold was sputtered with a thickness of 100 nm onto a glass substrate through an adhesive layer of chromium with a thickness of 3 nm and used as a working electrode. SAMs on a gold electrode was formed by immersing all substrates into a 1 mM (E)-2-(5-Mercapto-pentyl)-4-(phenyldiazenyl)phenol solution in ethanol for 48 h after cleaning in Piranha solution for 5 minutes. For the immobilization of proteins on the surface, the substrates were washed with absolute ethanol and dried under nitrogen stream three times, immersed in a 1 mM 3-mercapto-1-propanol solution for 3 h to passivate possible pinholes, washed again with absolute ethanol, and dried under nitrogen stream.

Electrochemical measurements. All experiments were performed using CHI-660A and CHI-601B potentiostats (CH Instrument, TX, USA). A home-made electrochemical cell was used to conduct electrochemical experiments, by using a platinum wire counter electrode and an Ag/AgCl reference electrode. The geometric area of the gold electrode exposed to the solution was 0.066 cm^2. The supporting electrolyte was Britton-Robinson buffer (40 mM boric acid, 40 mM acetic acid, and 40 mM phosphoric acid, pH 2.0 adjusted by adding sodium hydroxide) or deoxygenated phosphate buffered saline (PBS) (100 mM sodium phosphate, 150 mM NaCl, pH 7.4). All cyclic voltammograms (CVs) were obtained using scan rate of 50 mV/s.

Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS). ToF-SIMS spectra were obtained by using a ToF-SIMS V instrument (ION-TOF GmbH, Germany) with 25-keV Bi^+ primary ions. The primary ion source was operated with an average current of 0.95 pA, a pulse width of 13.7 ns, and a repetition rate of 10 kHz. Positive ion spectra were acquired on three points with an area of 200 × 200 μm^2 on each sample, while maintaining the primary ion dose of 5×10^{11} ions cm^{-2}. The positive and negative ion spectra were calibrated using the CH_3^+, $C_2H_3^+$, $C_3H_5^+$, Au^+, Au_2^+, Au_3^+ and CH^-, C_2H^-, C_4H^-, Au^-, Au_2^-, Au_3^- peaks, respectively. The spectra were normalized by the corrected total ion intensities, and the intensities of each molecular ion peak were compared. The mass resolution M/ΔM was more than 8000 between m/z 800 and m/z 1000.

Conjugation of cysteinyl biomolecules. For the cleavage of *p*-hydroxyazobenzene, a reductive potential of −0.3 V for 30 s was applied in Britton-Robinson buffer (B-R, pH 2.0), followed by an oxidative potential of 0.3 V for 60 s to yield *p*-quinoneimine in the presence of cysteinyl biomolecules in PBS. Cys and other control amino acids including Gly, Ser, Thr, His, and Lys were dissolved in borate buffer (pH 9.5) with final concentration of 50 mM. Laminin peptide CGG-IKVAV was prepared as 5 mg/ml (6.7 mM) stock solution in borate buffer. The cysteinyl protein β-lactoglobulin and cysteine-free myoglobin were dissolved in PBS with the final concentration of 1.0 mg/ml. To this was added TCEP (final 0.1 mM) to keep free thiols from forming disulfide bonds at high pH.

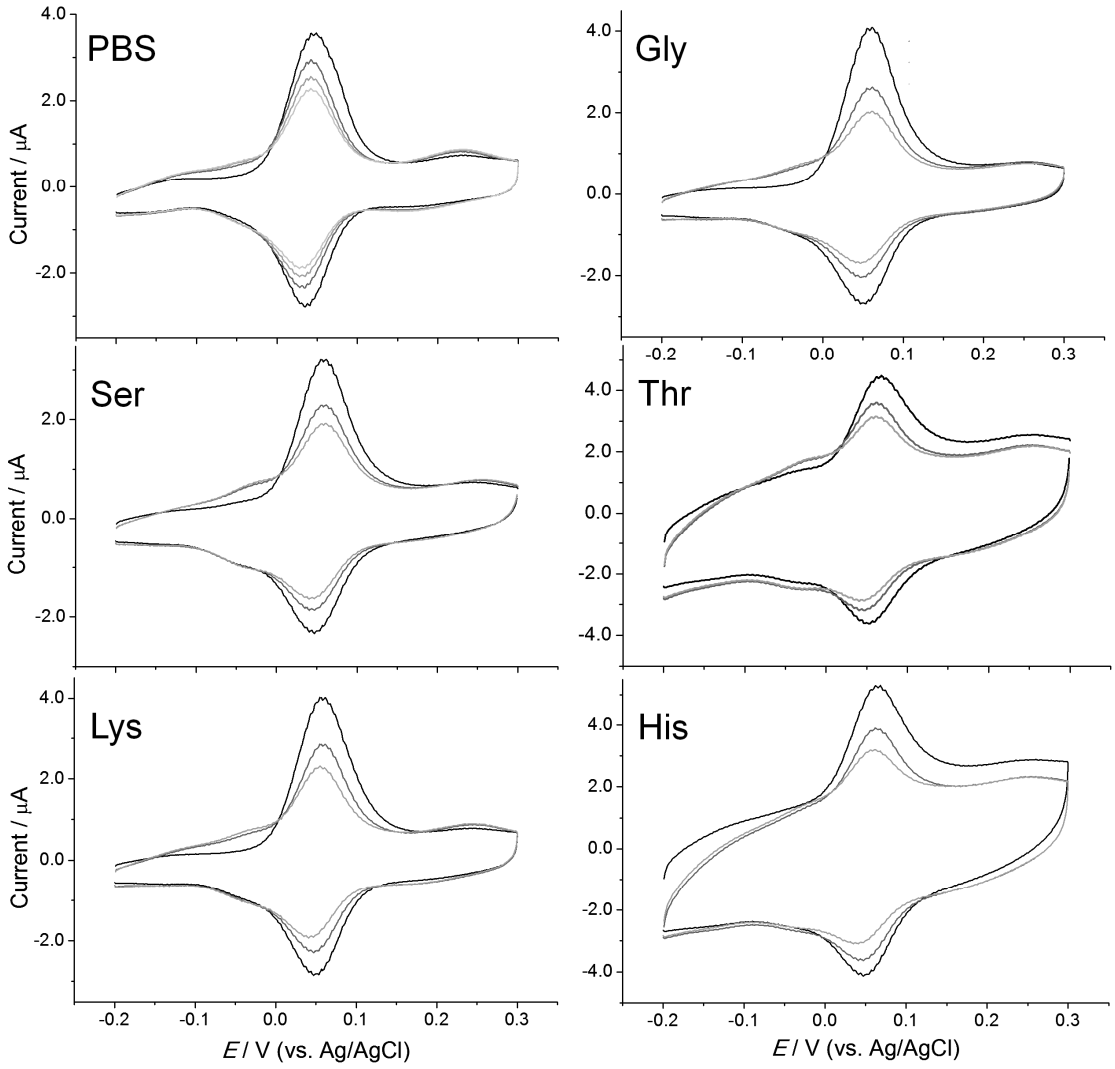


Figure S1. CVs of other amino acids including PBS buffer as a control. The reductive cleavage of *p*-hydroxyazobenzene was verified before the immobilization in each experiment (black). Various amino acids were then added to be a final concentration of 1.0 mM (dark gray) and 2.0 mM (gray). For the control, only PBS buffer was added (top left panel). No significant shift of peak potential was observed. The decrease in peak current as a result of repetitive scans is supposedly ascribed to degradation of the unstable oxidized quinoneimine.

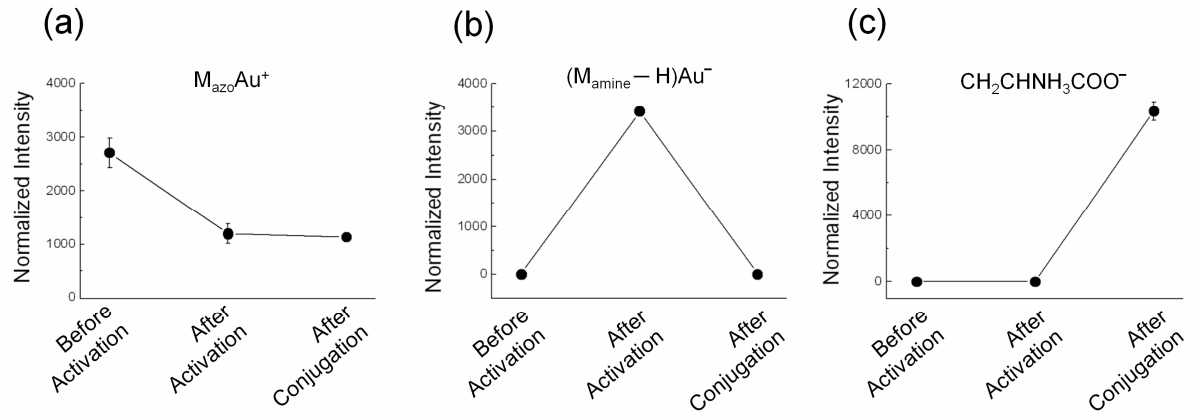


Figure S2. The normalized Tof-SIMS peak intensities of (a) *p*-hydroxyazobenzene (M_{azo}), (b) *p*-aminophenol (M_{amine}), and (c) cysteine fragment ($CH_2CHNH_3COO^-$) obtained from three independent spots of the SAM-modified electrode surfaces before and after the electrochemical activation and after the conjugation.

Table S1. Band assignments of FT-IR spectra.

Absorbance band (cm^{-1})	Vibration mode	Reference
3310	NH_2	
2920 / 2850	$CH_{2,asym}$ / $CH_{2,sym}$	
1715	NH_3^+,asym	
1652	NH_2	
1648	C=N	1
1596	φ–H	2
1512	NH_2	3
1481	φ–H	2
1395 / 1370	OCO	4
1265	φ–O	2, 5
1245 / 1156	φ–N	3

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