Electronic Supplementary Information (ESI)

Facile degradation of benzenediazonium-grafted thick layers on electrode surface enabling electrochemical biosensor application

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Experimental

Chemicals and instruments

Formyl-functionalized benzenediazonium tetrafluoroborate salt (FBD) was synthesized from 4-aminobenzaldehyde polymer (purchased from TCI, Japan) according to the literature cited.[S1] Mouse IgG, anti-mouse IgG, HPR-conjugated anti-mouse IgG and rabbit IgG were purchased from Sigma Aldrich. Phosphate buffer (PB, 0.1 M, pH 7.2) solution consisted of 0.1 M NaH_2PO_4 and Na_2HPO_4. Phosphate buffered saline (PBS, 0.1 M, pH 7.2) solution consisted of PB and 0.15 M NaCl. PBST consisted of PBS and 0.05% Tween 20. All buffers and aqueous solutions were made with 18.2 MΩ nanopure water. CV and EIS were carried out with Compactstat (Ivium, Netherlands). XPS was carried out with SIGMA PROBE (Thermo VG, U.K.). AFM was carried out with SPA-400 (Seiko Instrument). ATR-FTIR was carried out with Vertex 80 V spectrometer (Bruker optics).

Deposition and degradation of FBD-grafted organic layers

ITO electrodes were cleaned successively with acetone, ethanol, and piranha solution (H_2SO_4 : H_2O_2 = 3:1). Organic layers were formed by electrodeposition of FBD onto cleaned ITO surfaces using a solution of 2 mM FBD salt with 0.1 M tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) in acetonitrile (ACN). A classic three-electrodes electrochemical cell was used with the ITO electrodes as working electrode in connection to a $Ag/AgNO_3$ and Pt wire as a reference and counter electrode respectively. Three consecutive cycles were carried out from +0.2 to -0.8 V at a scan rate of 50 mV/s. For degradation of the electrodeposited organic layers, the electrodes were immersed in PBST for 3 h. This was followed by washing with PBST and water and dried under a stream of N_2 gas.

Preparation of immunosensing layers and enzyme-linked immunosorbent assay

Anti-mouse IgG solution (100 µg/mL in PBST) was dropped on the degraded surface for 2 h. Then the surface was incubated with the solutions of 1% BSA and (0.1 M ethanolamine + 5 mM $NaBH_3CN$) in PBS for 1 h each to block the nonspecific binding sites and excess aldehyde groups on the surface followed by washing with PBS and water and drying under a stream of N_2 gas. Then mouse IgG solution (different concentrations in PBST) was dropped on the surface and incubated for 1 h. Afterward, the surface was incubated with HRP-conjugated anti-mouse IgG solution (20 µg/mL in PBST) for 1 h. All antibody and antigen immobilization steps were followed by washing with PBST and water and drying under a stream of N_2 gas. For negative control experiment, same procedure was followed

without using any antigen. To test the specificity of the immunosensor, rabbit IgG was used in place of mouse IgG. All steps were carried out at room temperature.

Electrochemical detection of antigen

For the electrochemical detection of mouse IgG, the cell was filled with freshly prepared PBS solution of 1.5 mM H_2O_2 and 2.0 mM hydroquinone (HQ) and kept undisturbed during the enzymatic reaction for 5 min at room temperature. Then cyclic voltammetric measurements were carried out between 0.0 and -0.8 V at a scan rate of 50 mV/s and the reduction peak currents from the enzymatic reaction product BQ were recorded.

Reference

S1. L. L. Lai, C. H. Ho, Y. J. Lin, E. Wang, *Helv. Chim. Acta.* 85 (2002), 108-114.

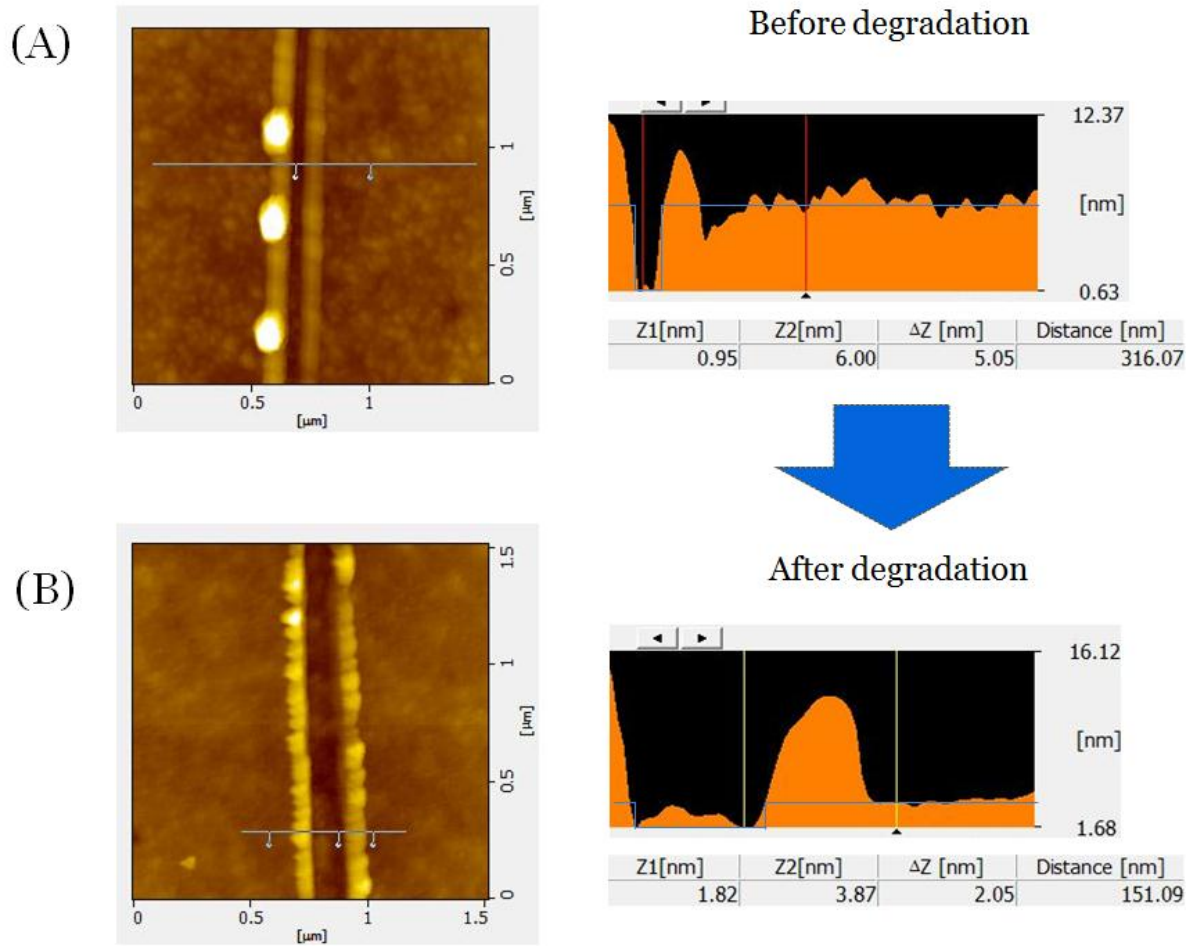

Figure S1. AFM images and line profiles obtained with the scratching method for FBD-deposited organic layers (A) before and (B) after the PBST treatment for 3h.

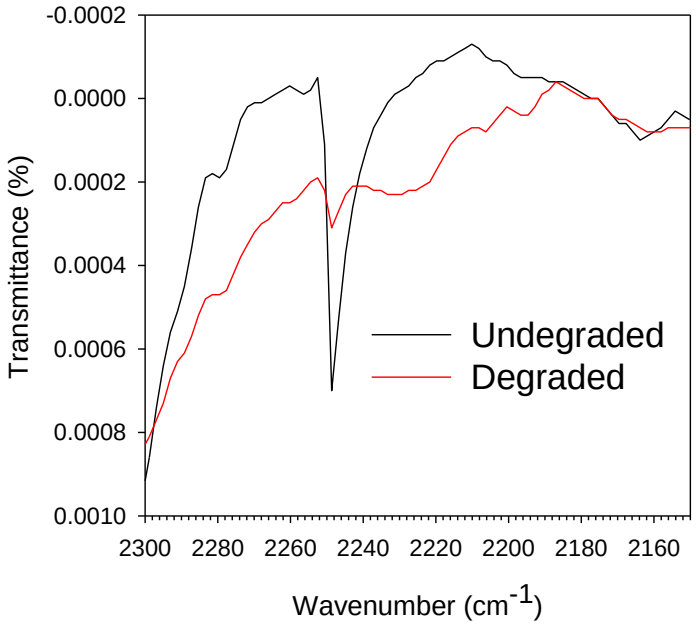

Figure S2. ATR-FTIR results before and after the degradation with PBST treatment for 3h.

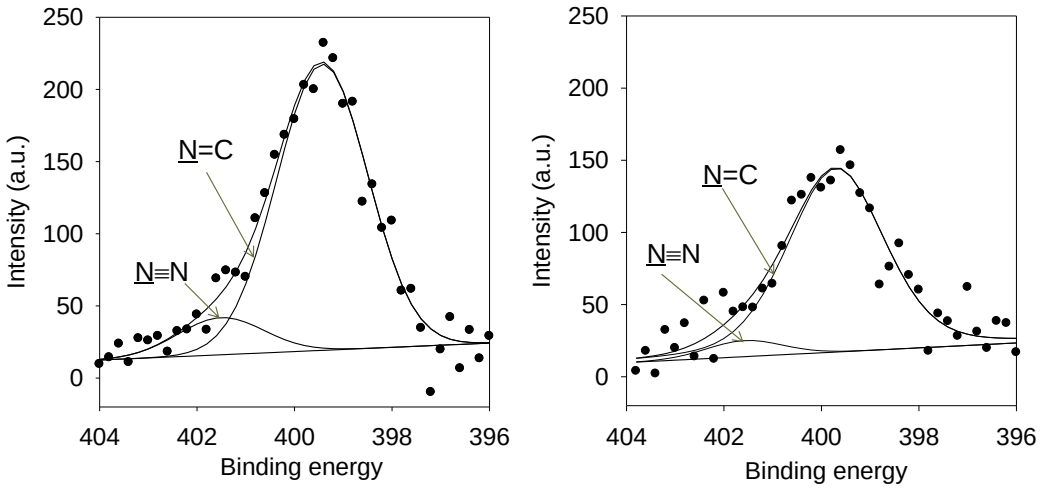

Figure S3. XPS spectra of N1s (left) before and (right) after PBST treatment of the FBD-deposited ITO surfaces.

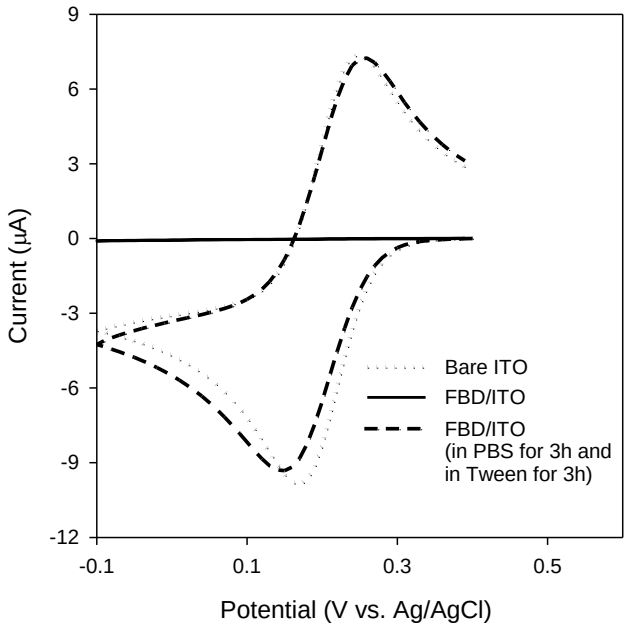

Figure S4. CVs for bare and FBD-deposited ITO surfaces recorded in 1 mM $[Fe(CN)_6]^{3-}$/0.1 M KCl solution. Scan rate of 50 mV/s.

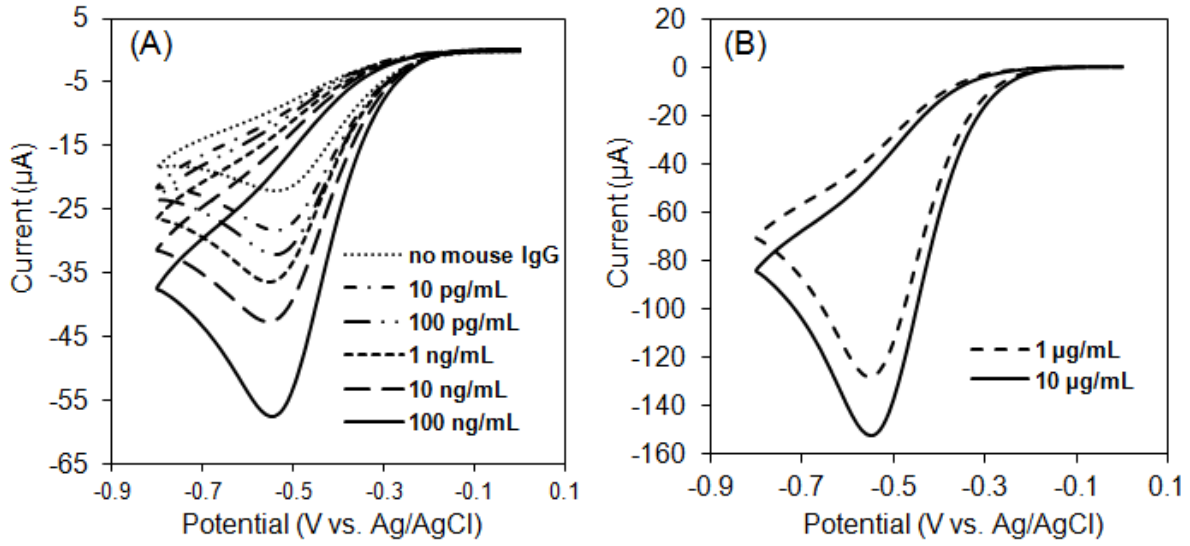

Figure S5. Cyclic voltammetric responses of the immunosensor for the detection of mouse IgG at concentrations from 10 pg to 100 ng (A), and 1 and 10 μg/mL (B), recorded in 0.1 M PBS (pH 7.2) containing 2 mM HQ and 1.5 mM H_2O_2 at a scan rate of 50 mV/s.

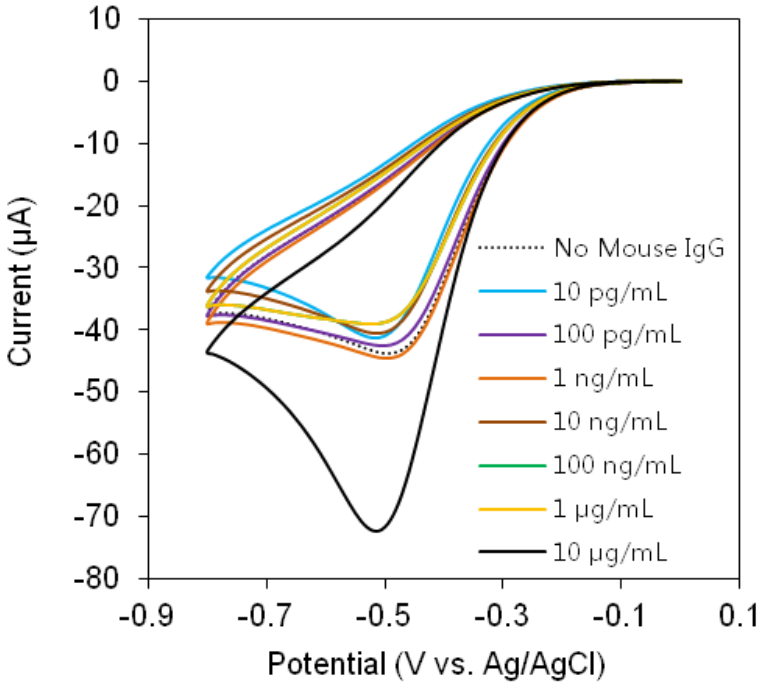

Figure S6. Cyclic voltammetric responses of the immunosensor on the undegraded surfaces after the detection of mouse IgG at different concentrations from 10 pg/mL to 10 μg/mL, recorded in 0.1 M PBS (pH 7.2) containing 2 mM HQ and 1.5 mM H_2O_2 at a scan rate of 50 mV/s.

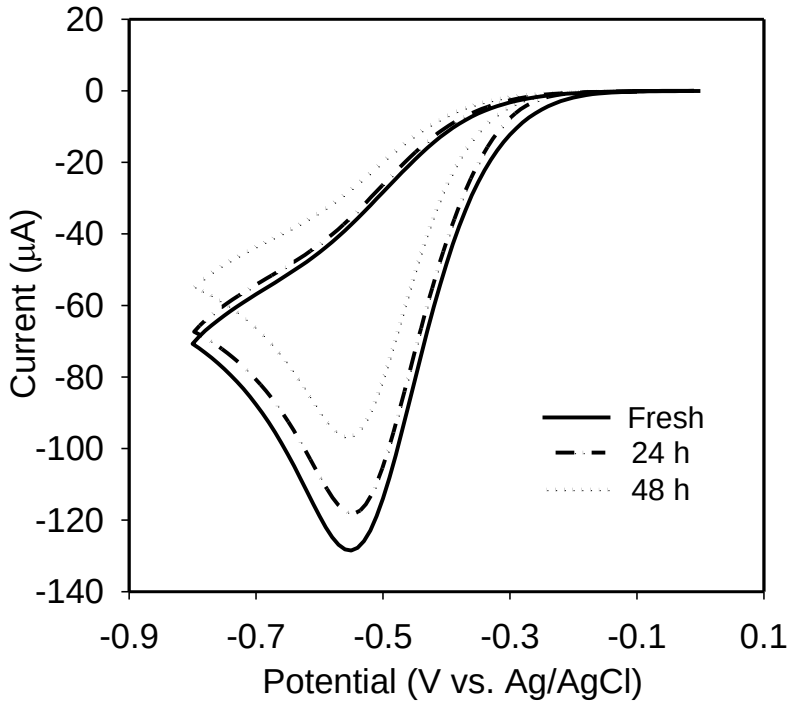

Figure S7. Storage time-dependent cyclic voltammetric responses of the immunosensor for the detection of mouse IgG at concentrations of 1 μg/mL, recorded in 0.1 M PBS (pH 7.2) containing 2 mM HQ and 1.5 mM H_2O_2 at a scan rate of 50 mV/s.