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Electronic Supplementary Information

Reusable Bio-Functionalized Surfaces Based on Electrochemical Desorption of Benzenediazonium-Grafted Organic Layers

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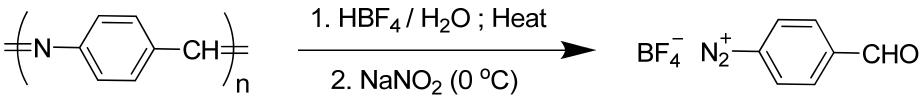
1. Reagents and apparatus

4-Aminobenzaldehyde polymer was purchased from TCI, Japan. H_2SO_4, H_2O_2 (30% in water), HBF_4 (50wt% in water), sodium nitrite ($NaNO_2$), tetrabutylammonium tetrafluoroborate (Bu_4NBF_4), potassium chloride (KCl), potassium ferricyanide, acetone, ethanol, acetonitrile (ACN), diethyl ether (Et_2O), rabbit IgG, anti-rabbit IgG, mouse IgG, anti-mouse IgG, TRITC labeled anti-rabbit IgG, FITC labeled anti-mouse IgG, were purchased from Sigma Aldrich. Phosphate buffered saline (PBS), PBST (PBS with 0.05% tween20). All buffers and aqueous solutions were made with 18.2 MΩ nanopure water. Fluorescence images were taken by Olympus-IX71 Fluorescence Microscope. Cyclic voltammetry were carried out with Ivium Compactstat (B07014) potentiostat interfaced with a PC computer. XPS was carried out with SIGMA PROBE (Thermo VG, U.K).

2. Synthesis of 4-formylbenzenediazonium tetrafluoroborate.



Scheme S1. Synthesisof 4-formylbenzenediazonium tetrafluoroborate.

The formylbenzenediazonium salt was synthesized according to the literature cited.[1] Briefly, 4-aminobenzaldehyde polymer (1.21 g, 10 mmol) was added to HBF_4 (8 mL), which was then diluted with hot H_2O (ca. 90 ^{0}C; 7 mL) and the solution was heated until the polymer completely dissolved and then allowed to cool to room temperature and finally cooled in an ice bath. $NaNO_2$ (1.03 g, 15 mmol) was gradually added at 0 ^{0}C, and the solution was then kept in the refrigerator overnight. Diazonium salt, which had precipitated, was filtered off, washed with dry Et_2O and then dried under vacuum to give quant. yield. The product was recrystallized by dissolving in cold acetonitrile followed by slow addition of cold dry Et_2O to recover the pure formylbenzenediazonium tetrafluoroborate salt.

References

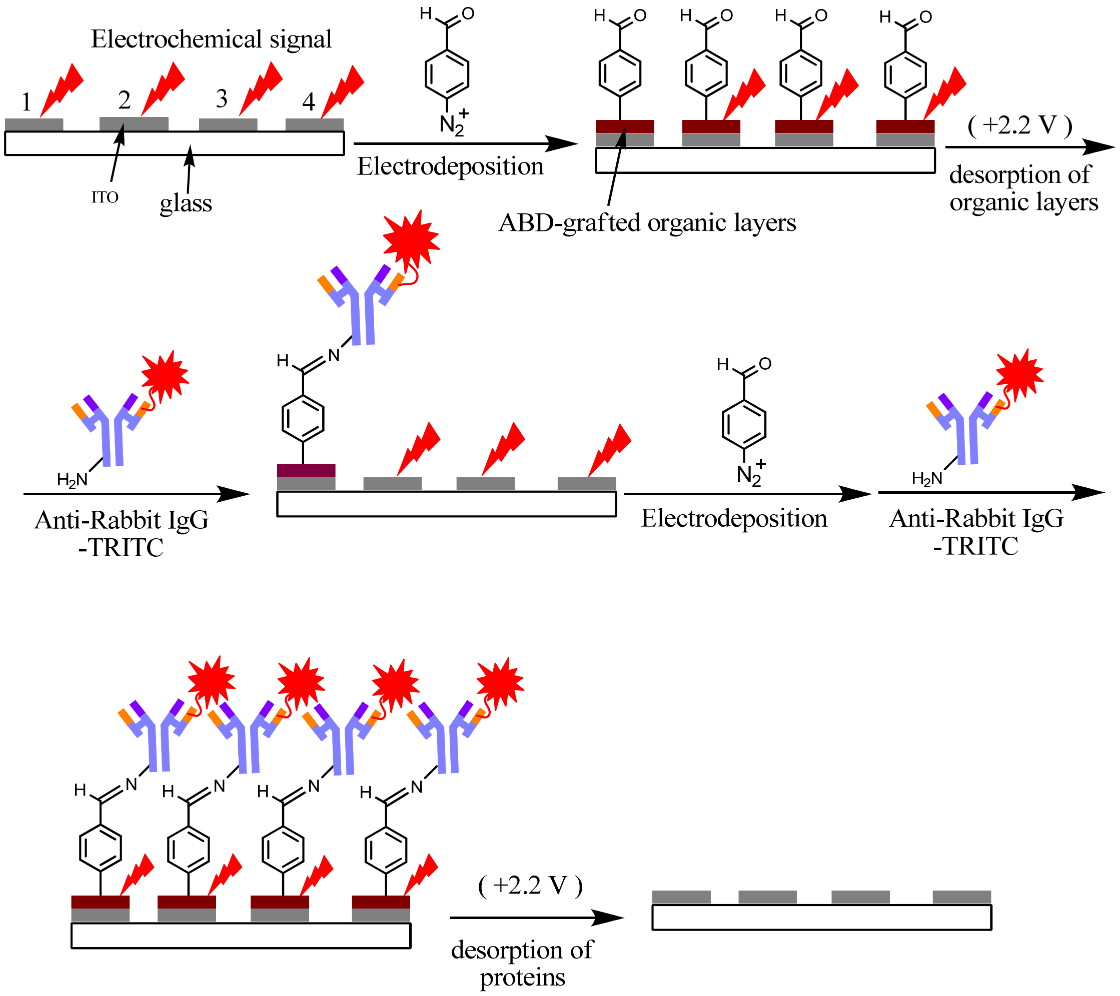
1. L. L. Lai, C. H. Ho, Y. J. Lin, E. Wang, Y. H. Liu, Y. Wang, Y. C. Lin, K. L. Cheng, *Helv. Chim. Acta,* 2002, **85**, 108-114.

3. Electrodeposition and oxidative desorption of organic layers

Prior to the surface modification, ITO electrodes were cleaned by rinsing with acetone, ethanol and water. After drying with N_2 gas, the electrodes were dipped in piranha solution (H_2SO_4 : H_2O_2 = 3:1) for several couples of seconds and finally washed with copious amount of water and dried under a stream of N_2 gas. [*Caution: Piranha solution should be handled with extreme care; it is a strong oxidant and reacts violently with many organic materials*]. Organic layers were formed by electrodeposition of ABD onto cleaned ITO using a solution of 2 mM ABD salt with 0.1 M Bu_4NBF_4 in acetonitrile. The ABD solution was deaerated with N_2 gas for 15 min before electrochemical reaction. 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 +0.3 to -0.7 V at a scan rate of 50 mV/s. After electrodeposition, the electrodes were rinsed with ACN and water and dried under a stream of N_2 gas. Oxidative desorption of electrodeposited organic layers on ITO electrodes was carried out using three-electrodes electrochemical cell with the ITO electrode as working electrode in connection to a Ag/AgCl and Pt wire as a reference and counter electrode respectively. An oxidative potential of +2.2 V was applied against Ag/AgCl reference electrode using deaerated 0.1M KCl in water solution for 30 seconds and the surface was washed with water.

4. Immobilization and desorption of proteins

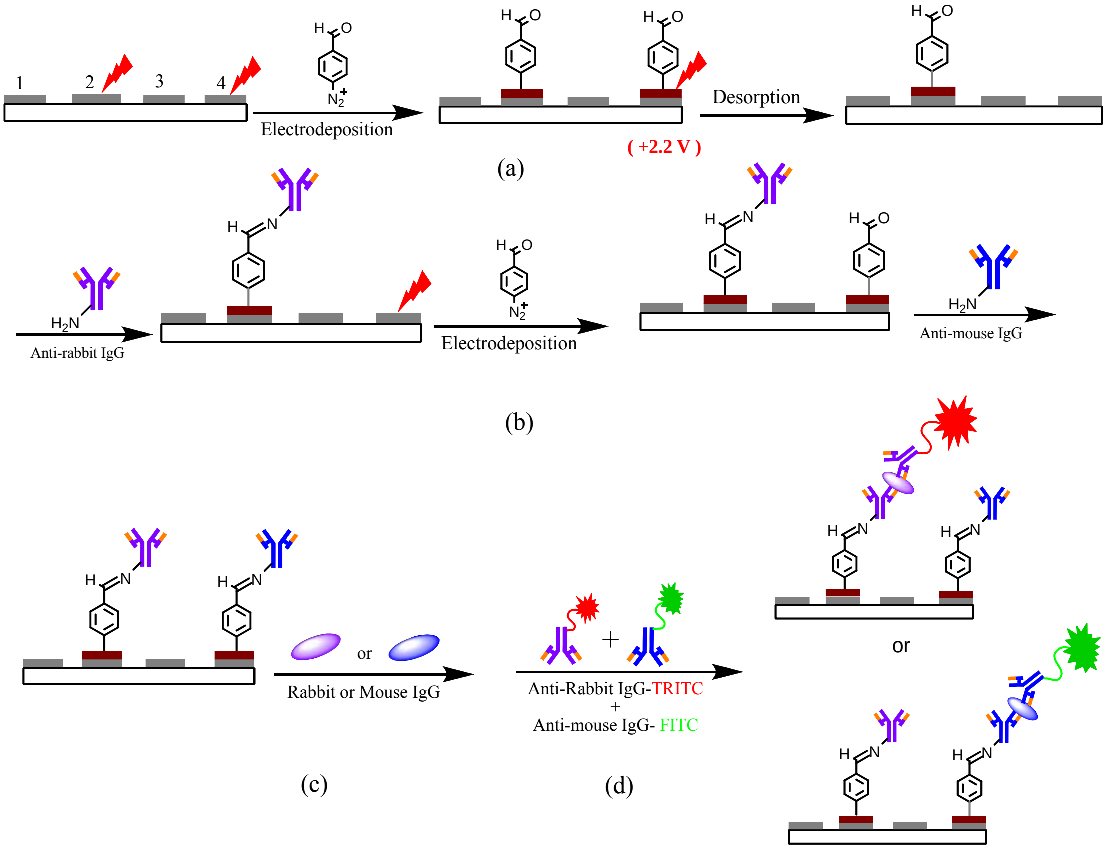
To immobilize protein on the surface, ABD deposited ITO electrode was incubated in antibody solution (50 µg/mL in PBST) for 2 h to covalently bind the protein through the coupling reaction of primary amine groups of protein and aldehyde groups present on the surface. The surface was then thoroughly rinsed with PBST and water and dried under a stream of N_2 gas. Oxidative desorption of the immobilized protein along with the organic layers was carried out using three-electrodes system with the protein immobilized ITO as working electrode in connection to a Ag/AgCl and Pt wire as a reference and counter electrode respectively. An oxidative potential of +2.2 V was applied against Ag/AgCl reference electrode using deaerated 0.1M KCl in water solution for 30 seconds and the surface was washed with PBST and water. The regenerated ITO electrode after desorption could be reused for immobilization and desorption of proteins with very good reproducibility up to several cycles.



Scheme S2. (A) Electrodeposition of ABD, desorption of organic layers, immobilization of anti-rabbit IgG, use of regenerated electrode surfaces and desorption of organic layers and proteins.

5. Sandwich immunoassay based on electrochemical desorption.

The procedure for the sandwich immunoassay is presented in Scheme S3. Two electrodes among four electrodes of the microarray were selectively functionalized with two different kinds of antibodies as probes. The protocol to prepare the platform for the immunoassay is summarized as a below. Following the simultaneous electrodeposition of ABD on electrodes '2' and '4', the electrode '4' only was addressed for the electrochemical desorption. The resulting array surface was exposed to a drop of anti-rabbit IgG solution of PBST for 2h. After washing of the exposed surface, ABD again electrodeposited on the electrode '4' to immobilize anti-mouse IgG following same procedure. For immunoassay, the platform of antibodies-immobilized array was incubated with a drop of 2 μg/mL mouse IgG in PBST solution for 1 h. After washing, the platform surface was exposed to a drop of mixture solution of anti-mouse IgG labeled with FITC and anti-rabbit IgG labeled with TRITC in PBST for 3 h. The same immunoassay procedure was repeated for rabbit IgG.



Scheme S3. (a) Electrodeposition of ABD and selective desorption of organic layers, (b) spatially selective immobilization of primary antibodies, (c) detection of antigen, (d) exposure to the fluorophore-labeled secondary antibodies.

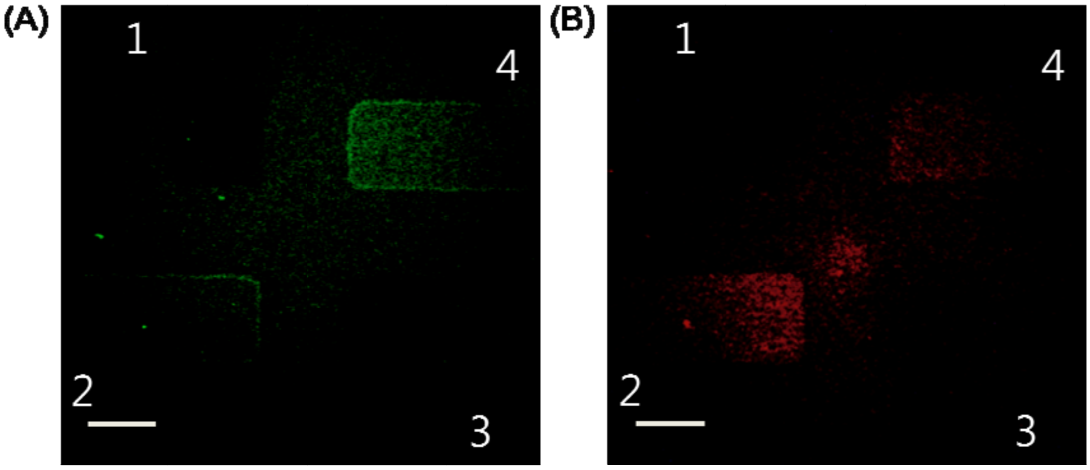


Figure S1. Fluorescence microscopic images after immunoassay for detecting (A) mouse IgG and (B) rabbit-IgG.