Electronic Supplementary Information

Use of 1,3-Dithiane combined with Aryldiazonium Cation for Immobilization of Biomolecules Based on Electrochemical Addressing

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1. Synthesis of 2-(4-Nitrophenyl)-1, 3-dithiane

![Scheme S1. Synthesis of 2-(4-Nitrophenyl)-1, 3-dithiane](image)

To a solution of 4-nitrobenzaldehyde (5 mmol), 1,3-propanedithiol (0.5 mL, 5 mmol), in CHCl₃ (25 mL) was added iodine (0.13 g, 0.5 mmol), and the resulting mixture was stirred at room temperature. After completion of the reaction (TLC, hexane/ethyl acetate, 2/1) the reaction was quenched with aqueous solutions of Na₂S₂O₃ (0.1 M, 25 mL) and NaOH (10%, 25 mL), respectively. Then CHCl₃ was added to the resulting reaction mixture. The organic layer was separated and washed with H₂O and decanted. The organic layer was dried over anhydrous MgSO₄ and filtered. Evaporation of the solvent gave the desired pure product in a high yield (58%). Further purification was achieved by recrystallization from Chloroform. 1H NMR (CDCl₃, 300Mz): δ ppm = 8.18-8.21(d,2H,Ar-H₂), 7.64-7.67(d,2H,Ar-H₂), 5.23(s, 1H, CH), 2.9-3.13(m,4H,CH₂), 2.19-2.23(m,1H,CH₂), 1.56-1.98(m,1H,CH₂). FTIR : ν (cm⁻¹) = 1540,1350 (NO₂), 1600 (Aromatic str.).
2. Synthesis of 2-(4-Aminophenyl)-1,3-dithiane

![Scheme S2. Synthesis of 2-(4-Aminophenyl)-1,3-dithiane](image)

2-(4-Nitrophenyl)-1, 3-dithiane (2.0 mmol) and SnCl₂·2H₂O (10.0 mmol) were dissolved in THF and ethanol (3/1, 40mL) and heated to 60 °C. Sodium borohydride (1.0 mmol) in ethanol (10 mL) was added over a period of 30 min with stirring. After being stirred for further 30 min, the reaction mixture was cooled to 5-10 °C and 40 mL of chilled water was added, followed by neutralization with chilled 3.5 N NaOH to pH 7. The THF and ethanol was evaporated off, and the aqueous solution was continuously extracted with ether. The ethereal solution was dried over anhydrous Mg₂SO₄ and concentrated to give a yellowish solid which was purified by column chromatography (yield 34%). ¹H NMR (CDCl₃, 300Mz): δ ppm = 7.24-7.26(d,2H,Ar-H), 6.61-6.14(d,2H,Ar-H), 5.08(s,1H,CH), 3.68(b,2H,NH), 2.84-3.09(m,4H,CH₂), 2.11-2.18(m,4H,CH₂), 1.92-1.92(m,1H,CH₂). FTIR: ν (cm⁻¹) = 3400,3350 (N-H, str), 1640 (N-H, bend).

3. Synthesis of 4-(1,3-Dithiane-2-yl)benzenediazonium chloride

![Scheme S3. Synthesis of 4-(1,3-Dithiane-2-yl)benzenediazonium chloride](image)

To make 1.5 mM 2 mL solution, 0.634 mg of 2-(4-Aminophenyl)-1,3-dithiane was dissolved in 1.9 mL 0.1 M HCl and cold in ice bath. Cold NaNO₂ (0.23 mg in 0.1 mL H₂O) solution was added dropwise with stirring. Stirring continued for more 30 min. Freshly prepared diazonium solution was used for electrodeposition.
4. Immobilization of biomolecules and a sandwich immunoassay

The procedure for the preparation of aldehyde group terminated electrode and subsequent selective immobilization of biomolecules is presented in Scheme S4. Two opposite electrodes of a microarray consisting of four closely spaced individually addressable ITO microelectrodes were selectively functionalized with two different kinds of antibodies to detect specific antigens. Following the electrodeposition on all of four electrodes, the electrode ‘1’ was deprotected and the array was treated with a 40 μL drop of 50 μg/mL anti-rabbit IgG solution in PBST and incubated for 2h at room temperature. This was followed by washing with PBST and water and dry with N₂ gas. After deprotection of electrode ‘3’, the array was treated with a 40 μL drop of 50 μg/mL anti-mouse IgG solution in PBST and incubated for 2h at room temperature. This was followed by washing with PBST and water and dry with N₂ gas. After deprotection of electrode ‘1’, the array was treated with a 40 μL drop of 50 μg/mL anti-rabbit IgG solution in PBST and incubated for 2h at room temperature. This was followed by washing with PBST and water and dry with N₂ gas. After deprotection of electrode ‘3’, the array was treated with a 40 μL drop of 20 μg/mL mixture solution of TRITC labeled anti-rabbit IgG and FITC labeled anti-mouse IgG in PBST for 3h at room temperature. This was followed by washing with PBST and dry with N₂ gas. Fluorescence image (Figure 2a.) shows only electrode ‘3’ of the ITO microarray with significant intensity of FITC and confirmed the selective immobilization of mouse IgG. Then the array was exposed to 40 μL drop of 20 μg/mL mixture solution of TRITC labeled anti-rabbit IgG and FITC labeled anti-mouse IgG in PBST for 3h at room temperature. This was followed by washing with PBST and dry with N₂ gas. The array was exposed to 40 μL drop of 20 μg/mL mixture solution of TRITC labeled anti-rabbit IgG and FITC labeled anti-mouse IgG in PBST for 3h at room temperature. This was followed by washing with PBST and dry with N₂ gas. Fluorescence image (Figure 2b) shows only electrode ‘1’ of the ITO microarray with significant intensity of TRITC and confirmed the selective immobilization of rabbit IgG.
Scheme S4. (a) Electrodeposition of 4-(1,3-dithiane-2-yl)benzenediazonium cation, (b) spatially selective immobilization of primary antibodies, (c) detection of antigen, (d) exposure to fluorophore-labeled secondary antibodies.
5. XPS data for sulfur (2p)

![XPS data for sulfur (2p)](image)

*Figure S1. XPS data for sulfur (2p)*