Supporting Information

Electrochemical thrombin detection based on the direct interaction of target protein and graphene oxide as an indicator

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Reagents and apparatus

All chemicals including graphite powder (<20 μm) were purchased from Sigma Aldrich. Thrombin (from bovine plasma), Alcohol Dehydrogenase (ADH), Streptavidin, Rabbit-igG, Myoglobin, Albumin form bovine serum (BSA) were purchased from Sigma Aldrich. TBA_{29} (SH-C_6-5' TTT TTA GTC CGT GGT AGG GCA GGT TGG GGT GAC T-3') was purchased from Genotech (Korea). Phsphate buffer (PB; pH 7.4, 0.1 M phosphate). Tris-HCl buffer (pH 7.4, 50 mM Tris-HCl , 140 mM NaCl , 1mM $MgCl_2$ and 0.1% BSA). All buffers were made with 18.2 MΩ pure water. All electrochemical measurements including cyclic voltammetry (CV) and EIS were carried out with Ivium Compactstat (B0714) as a potentiostat interfaced with a PC. Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-7001F. The Sonicated process was performed with the Ultrasonic processor (YUJINSM).

Synthesis of Graphene Oxide

Graphene oxide (GO) suspension was synthesized from graphite powder using a modified Hummers method. Nature graphite (3 g, 99.95% in purity) was mixed with 69 mL of concentrated sulfuric acid in a 500 mL Erlenmeyer flask and stirred at room temperature for 10 min. Next, 1.5 g of $NaNO_3$ was added to the mixture and allowed to dissolve for 20 min. The flask was then placed in an ice bath, and 9 g of $KMnO_4$ was slowly

added while the temperature was kept below 20 c for 1 h. The solution was then changed into the water bath and heated at 35 °C for 3 h. afterward 15 mL of water was added to the flask and this could increase the temperature to 100 °C. Keep the temperature for 20 min. Twenty minutes later another 15 mL of water was added. After 20 min, 100 mL of water was added. Then add 200 mL of ice water into the resultant suspension; this step can dilute and cool down the system to 40 °C. After 15 min, 3 mL of 30 % H_2O_2 was added to the flask under vigorous stirring. This suspension was stirred at room temperature for 30 min. The suspension was centrifuged at low speed for 7 times (4500 rpm, 15 min) with distilled water and 5 % HCl solution for 2 rounds and then centrifuged at high speed for 5 times (12000 rpm, 60 min) with distilled water and then purified by using dialysis sack for 1 week.

Immobilization of Thrombin-binding aptamer (TBA) on the gold surfaces.

First, gold coated glass electrodes were cleaned by using piranha solution (H_2SO_4:H_2O_2 = 3:1), washed with sufficient amount of water, and finally dried with N_2 gas. Prior to the surface modification, 10 nM TBA (SH-C_6-5' TTT TTA GTC CGT GGT AGG GCA GGT TGG GGT GAC T-3') was mixed with 0.1 M Phosphate buffer solution with 1 mM tris(2-carboxyethly)phosphine (TCEP) to reduce the disulfide bonds between TBA. The freshly cleaned gold electrodes were soaked in a mixture solution of TBA and TCEP for 2 h. After TBA immobilization, the electrodes were immersed in 1 mM 6-Mercapto-1-hexanol (MCH) for 1 h to reduce nonspecific adsorption, to ensure adequate space for TBA, and to remove loosely bond TBA.

Immobilization of Thrombin and GO on the modified electrodes.

The various concentrations of Thrombin (with concentration raged from 100 pg/ml to 1 µg/ml) in Tris-HCl buffer were added to the surface of the primary aptamer modified gold electrode for 1.5 h. And then, the resulting electrodes were immersed in diluted GO solution (0.1 mg/ml) for 50 min to obtain GO/Thrombin/TBA/Gold electrodes followed by washing with water and drying under a stream of N_2 gas. Prior to immersing the resulting electrode in GO solution, GO was sonicated for 1 h with ultrasonic processor and filtered with 450 nM cut-off membrane glass filter. Furthermore, pH of Go solution is adjusted with concentrated HCl or NH_4OH.

Electrochemical detection of Thrombin

Electrochemical measurements were carried out using Ivium Compactstat (B07014) potentiostat. An electrochemical cell consists of the modified gold electrode as a working electrode in connection to Ag/AgCl and Pt wire as a reference and a counter electrode respectively. EIS measurements were carried out for characterization of resulting surface at every step and recorded between 10 kHz and 0.05 Hz at the formal potential of $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1 mM, 1:1 molar ratio) in PB with 0.1 M KCl. For electrochemical reduction of GO-modified surface, a reductive scan was started from 0.0 V to -1.2 V in a solution of 0.5 M NaCl.