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Supplementary Information

A Simple Modular Aptasensor Platform Utilizing Cucurbit[7]uril and Ferrocene Derivative As an Ultrastable Supramolecular Linker

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Experimental

General Procedures.

All the reagents and solvents employed were commercially available and used as supplied without further purification. CB[7] was purchased from CBTech (www.cbtech.co.kr). Thrombin (human α thrombin) was purchased from Heamatologic Technologies. SPR chips (J1) were purchased from Biacore. Water was purified using a Millipore purification train. FT-IR spectra were recorded on a Perkin-Elmer Spectrum GX FT-IR spectrophotometer. Surface plasmon resonance (SPR) sensorgram were recorded on a BIAcore 2000. AFM images were collected using a Park System NX10. The aptamers (Bioneer, South Korea) used in this study have the following sequence: 15-mer thrombin aptamer: 5'-GGT TGG TGT GGT TGG-3'; amine terminated 15-mer thrombin aptamer: 5'-NH_2-GGT TGG TGT GGT TGG-3'; amine terminated 15-mer scrambled aptamer: 5'NH_2- GGT GGT GGT TGT GGT-3'.

Preparation of CB[7] SAM on gold surface by Surface Plasmon Resonance (SPR) instrument.

Surface plasmon resonance (SPR) experiments were carried out at 25°C using a Pioneer Sensor Chip J1 (a plain gold surface) with a size of 7 × 7 mm^2 as a substrate. The pre-cleaned and dried bare gold substrate was mounted on an SPR cartridge with double-sided tape. All solutions used for SPR measurements were freshly prepared, filtered through a 0.22 μm filter, and degassed in an ultrasonic bath prior to use. The running solution for the SPR spectrometer was deionized H_2O. The concentration of CB[7] was 1 mM in H_2O. The steps involved for recording of a sensorgram include; (1) flowing running solution over the gold surface to establish a baseline (flow rate 20 μL/min), (2) flowing the same running solution

containing CB[7] for 20 min to observe binding, and (3) rinsing the substrate with running solution to quantify the amount of the CB[7] immobilized. The steps (2) and (3) were repeated for 5 times to maximize the immobilization of CB[7]. The change in signal (1000 RU = 1 ng/mm^2) was recorded as a function of time as shown in Fig. S1.

Immobilization of FA-TBA and FA-SDNA on CB[7] SAM and sensing thrombin or BSA using FA-TBA@CB[7] SAM sensor chip.

SPR experiments were performed at 25°C in filtrated degassed phosphate buffered saline (PBS). The solution of FA-TBA, FA-SDNA, and TBA (76 μM) was prepared in PBS and injected into the each channel on the CB[7] SAM-decorated gold chip mounted in SPR instrument for 20 minutes at flow rate of 5 μL/min, followed by washing with the PBS buffer. Thrombin (10 μg/mL) or BSA (10 μg/mL) was prepared in PBS containing 0.005%w/v sodium dodecylsulfate (SDS) and injected into the SPR instrument for 10 minutes at flow rate of 5 μL/min (Fig. S3). To observe the sensor response as a function of thrombin concentration, solutions of thrombin (0.1, 0.5, 1.0, 2.5 μg/mL) in PBS containing 0.005%w/v SDS were injected for 15 minutes at flow rate of 5 μL/min.

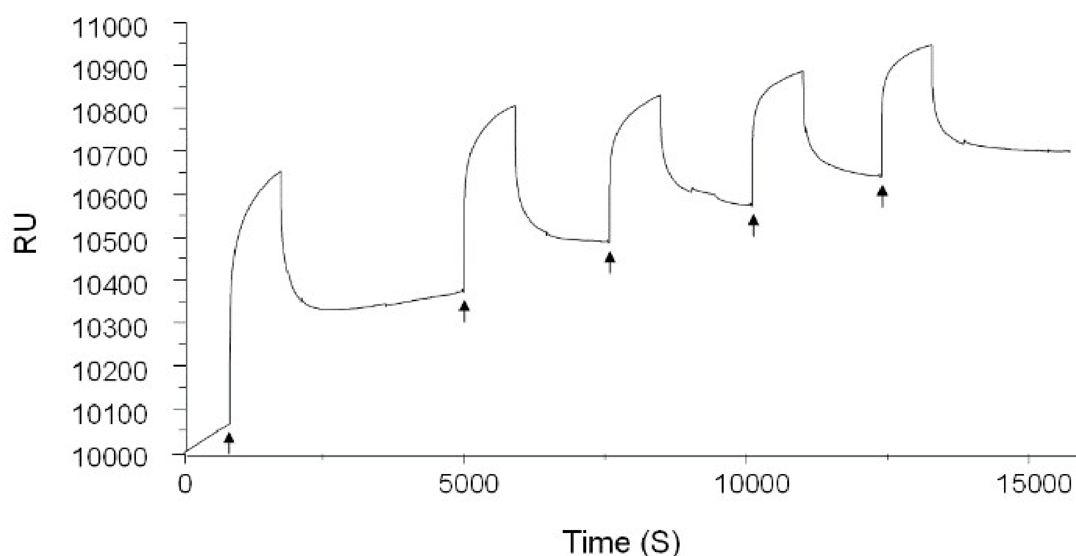
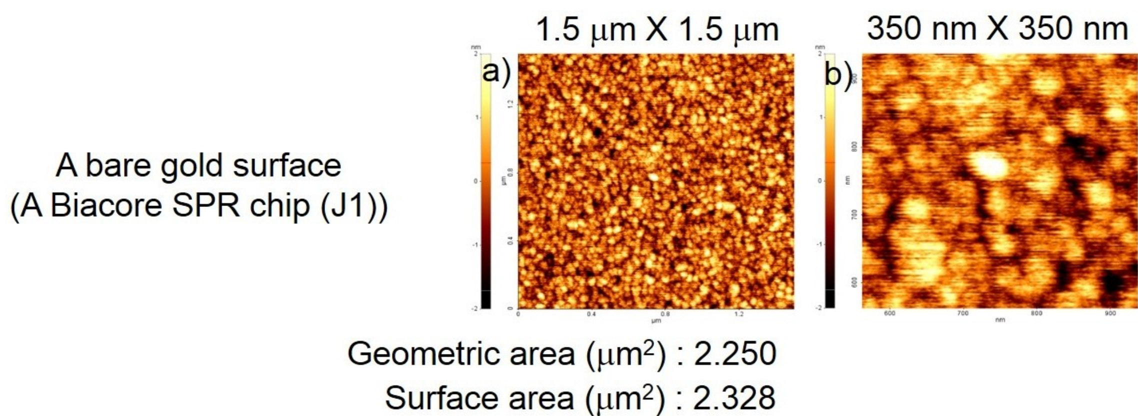


Figure S1. Immobilization of CB[7] on a SPR chip (Arrows indicate injection of a solution of CB[7])



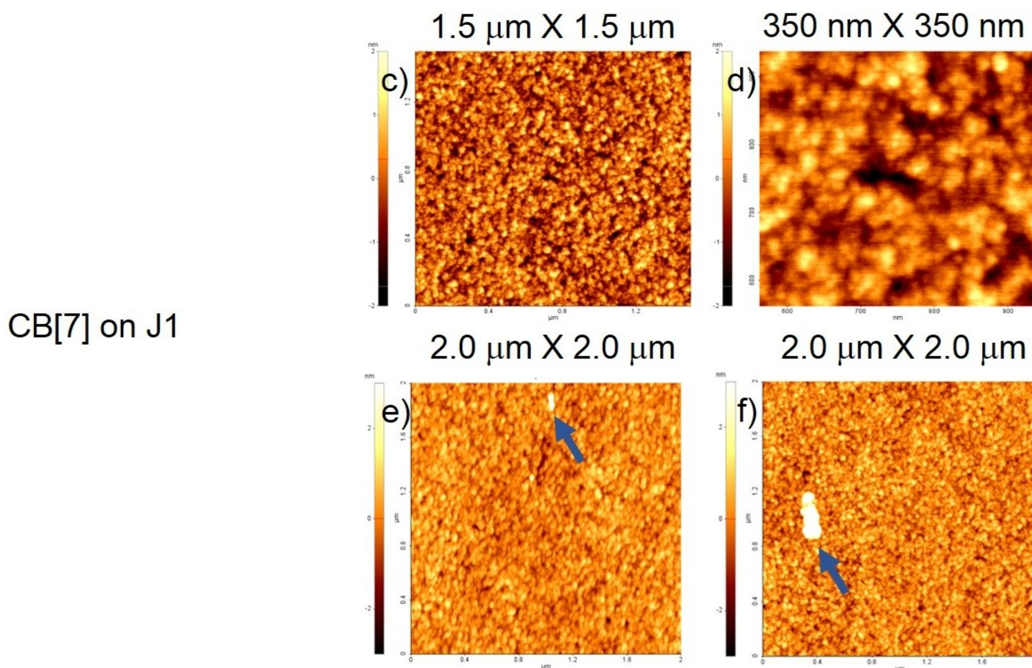


Figure S2. AFM images of a gold surface (J1) a) and b) before and c), d), e) and f) after treatment of CB[7] (The arrows indicate aggregation of CB[7]). Geometric area: plain area.[S1] Surface area: Supposed surface area as 1 by 1 pixel which is an area composed with four different points (Z1, Z2, Z3 and Z4). Z5 is calculated as average height value from Z1 to Z4 and located in the middle of them. Now, there are four surface (A1, A2, A3 and A4), which added up for area of 1 by 1 pixel as shown in Fig. S3.[S1]

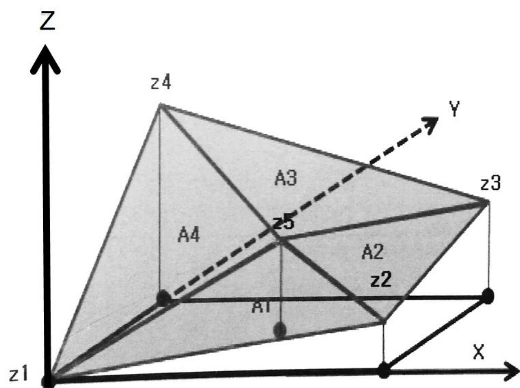
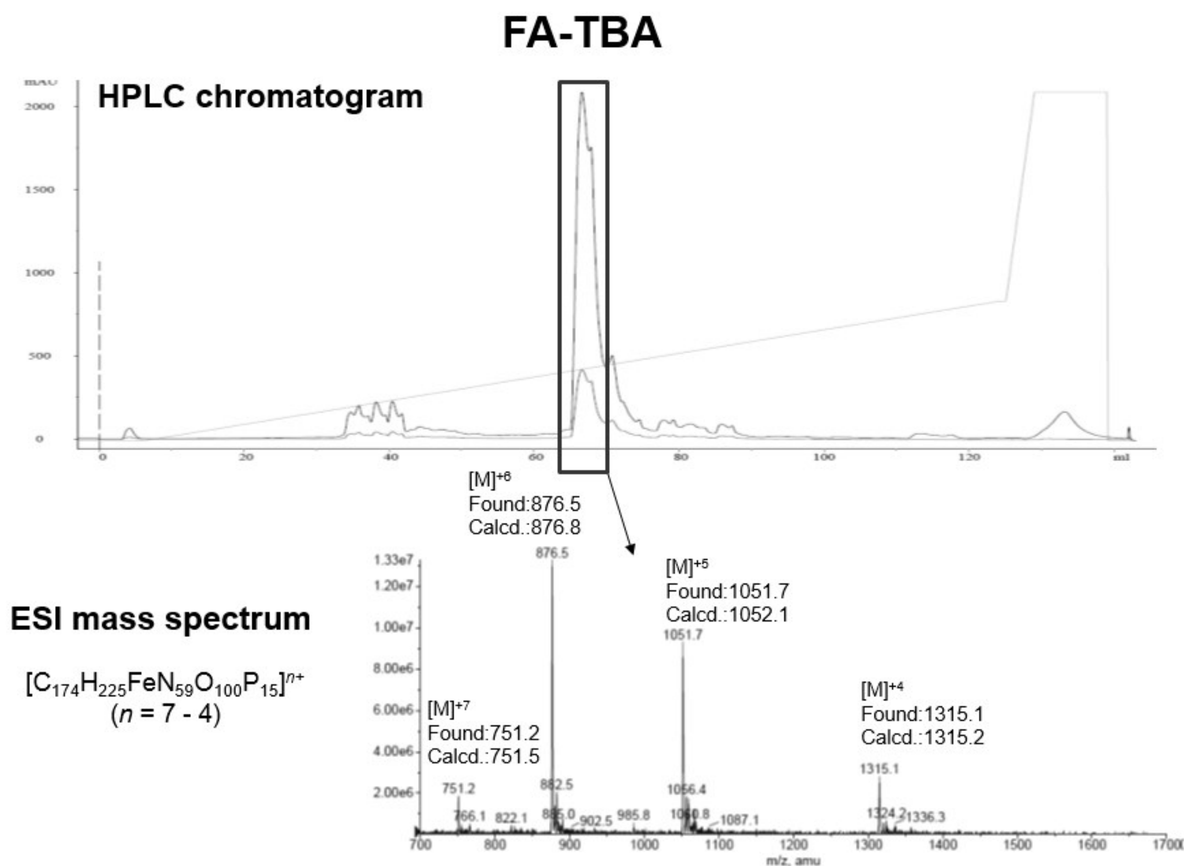


Figure S3. 1 by 1 pixel surface area[S1]

Ferrocenylation of thrombin binding aptamer (TBA) and scrambled DNA-aptamer (SDNA).

EDC (0.3 mg, 1.6 μmol) and NHS (0.2 mg, 1.7 μmol) were added to a solution of ferroceneaminetetraethyleneglycol-carboxylic acid (0.5 mg, 1.3 μmol)[S2] in dimethylformamide (DMF, 20 μL) and the reaction mixture was stirred at room temperature for 2 h. The crude product was directly added to a solution of amine terminated 15-mer thrombin aptamer or scrambled aptamer (100 μL, 0.2 nmol/μL in H_2O) and the mixture was stirred for 1 h at RT. The crude resulting mixtures were subjected to HPLC purification to give pure ferrocene labeled TBA (FA-TBA) and scrambled DNA sequences (FA-SDNA), which were analyzed by ESI-mass spectrometry. HPLC chromatograms and ESI-MS spectra for FA-TAB and FA-SDNA are shown in Fig. S4



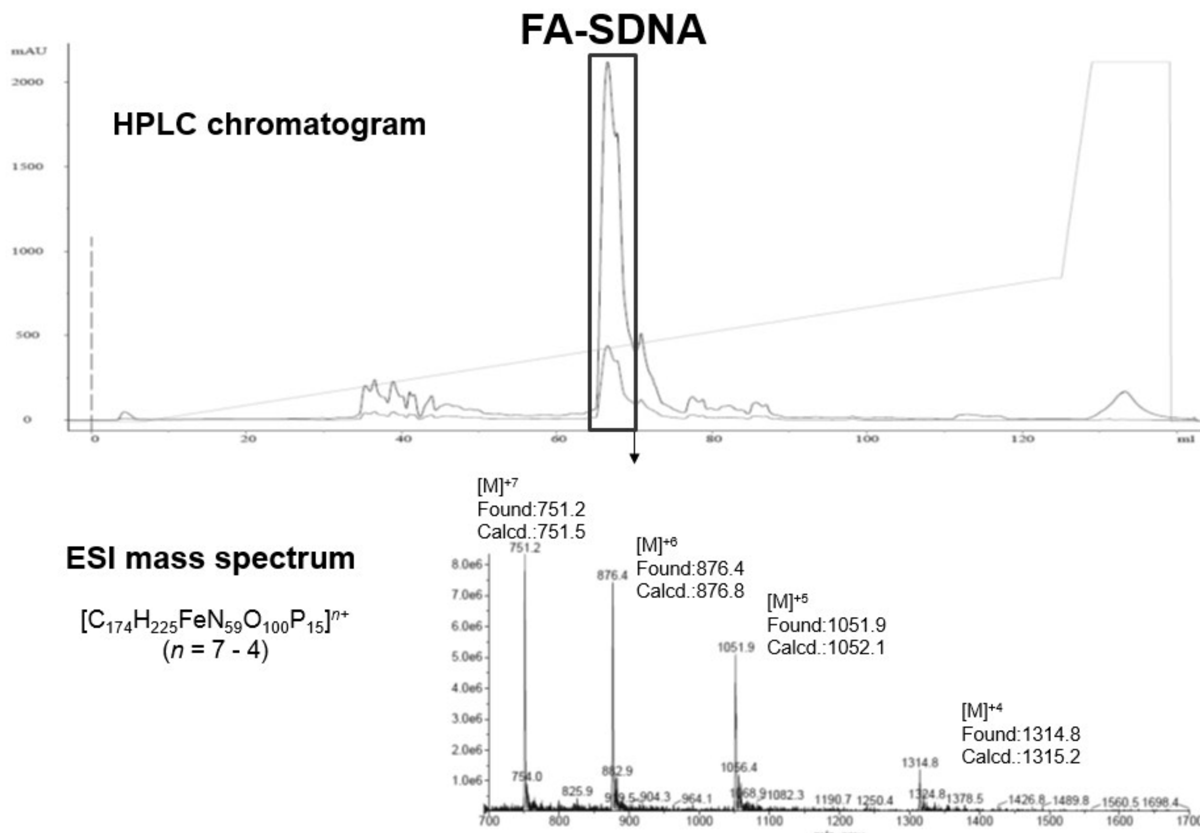


Figure S4. HPLC chromatograms and ESI mass spectra of FA-TBA and FA-SDNA

Determination of the binding constant of FA-TBA to thrombin.

The affinity of the FA-TBA for thrombin was determined by performing a filter binding assay[S3] with increasing amounts of a target protein and a constant amount of the aptamer. The 3'-terminal of FA-TBA was labeled with α-P^{32}ATP (Perkin Elmer). The α-P^{32}ATP-labeled aptamer was heated to 95°C and slowly cooled (0.1°C/sec) to 37°C with HEATCOOL protocol on thermalcycler. Thrombin was diluted into $SB17T_{0.002}$ to give a series of concentrations of thrombin (0.8, 1.6, 3.2, 6.2, 12.5, 25, 50 and 100 nM) that were incubated with α-P^{32}ATP-labeled FA-TBA in a selection buffer ($SB17T_{0.002}$) at 37°C for 0.5 h. After the binding reaction, the sample (5 μL) was spotted onto a Nylon membrane (GE healthcare) for input and Zorbax resin (Agilent) was added to the reaction tubes with mixing by aspiration. Then, the sample was filtered over a Durapore filter (Millipore) and washed with $SB17T_{0.002}$ to remove unbound aptamers. After a Nylon filter and a Durapore plate were exposed on a phosphorimager screen overnight, the screen was read on the phosphorimager (FLA-5100, Fuji). The amount of protein-bound aptamers was calculated and plotted at each protein concentration. On the basis of these data, a saturation curve was obtained and the dissociation constant K_d was calculated by non-linear regression analysis (SigmaPlot 11(Systat Software Inc.)) and converted to binding constant.

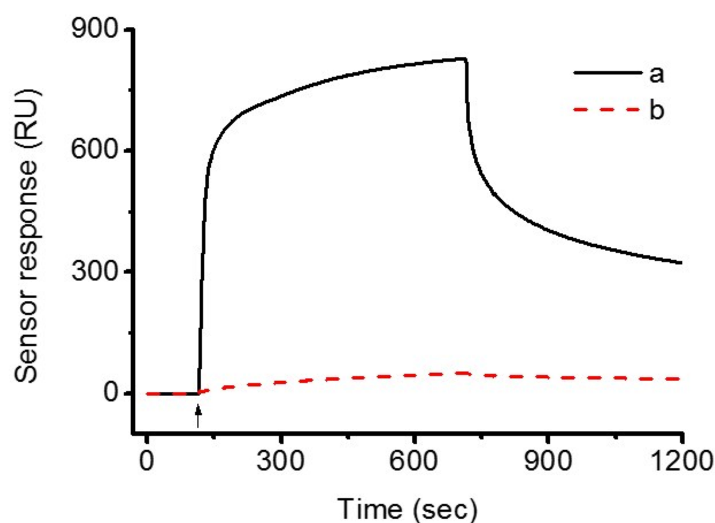


Figure S5. Sensorgrams of a) thrombin and b) BSA injected to FA-TBA@CB[7] SAM sensor chip. Each protein sample was injected for 10 min in the concentration of 10 μg/mL with flow rate of 5 μL/min (The arrow indicates injection of samples)

References

S1 XEI Powerful Image Processing Tool for SPM data, Software Manual (Version 1.8.0), Park SYSTEM.

S2 D. W. Lee, K. M. Park, M. Banerjee, S. H. Ha, T. Lee, K. Suh, S. Paul, H. Jung, J. Kim, N. Selvapalam, S. H. Ryu and K. Kim, *Nature Chem.*, 2011, **3**, 154-159.

S3 R. Conrad and A. D. Ellington, *Anal. Biochem.*, 1996, **242**, 261-265.