Supporting information

Surface Plasmon Coupled Fluorescence-Enhanced Interfacial "Molecular Beacon" to Probe Biorecognition Switching: An Efficient, Versatile, and Facile Signaling Biochip

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Chemicals and materials

2-morpholinoethanesulfonic hydrate (MES), glycine, cysteamine, mercaptoacetic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), poly (sodium 4-styrenesulfonate) (PSS), poly (allylamine hydrochloride) (PAH), 1-pyrenebutyric acid, glutaric anhydride, Rhodamine 6G, guanidine hydrochloride, PBS buffer, and thrombin was purchased from Sigma-Aldrich. 3-aminopropyltriethoxysiane was purchased from Acros Organics. N,N-dimethylformamide (DMF) and ethanol was purchased from Sinopharm Chemical Reagent Co., Ltd. Cell growth medium was purchased from Shanghai Basal Media Technologies Co., Ltd. Graphene oxide solution (2 mg/mL) was purchased from XFNANO (Nanjing, China). All materials were used as received without any further purification.

DNA sequences were purchased from Talara biotechnology (Dalian) Co., Ltd., and were prepared in 10 mM PBS buffer (pH=7.4). The sequence of these DNAs are: (1) for protein detection: 5'-NH_2-GGTTGGTGTGGTTGGTTTT-Texas Red-3'; (2) for 8 bases DNA detection: 5'-NH_2-GTTCCGTC-Texas Red-3' (probe); 5'-GACGGAAC-3' (target); (3) for 16 bases DNA detection: 5'-NH_2-CGGATCTCGATGAGCT-Texas Red-3' (probe); 5'-AGCTCATCGAGATCCG-3' (target); (4) for 24 bases detection: 5'-NH_2-GTTAGGAAAAAATCAAACACTCGC-Texas Red-3' (probe); 5'-GCGAGTGTTTGATTTTTTCCTAAC-3' (target); (5) for 48 bases detection: 5'-NH_2-CGCTCTGGAAATGTTCAATGAGGACTATGTGACATTCCCCAGGGACGC-Texas Red-3' (probe); 5'-GCGTCCCTGGGGAATGTCACATAGTCCTCATTGAACATTTCCAGAGCG-3' (target); (6) for HIV detection: 5'-NH_2-TGCATCCAGGTCATGTTATTCCAAATATCTTCT-Texas Red-3' (probe); 5'-AGAAGATATTTGGAATAACATGACCTGGATGCA-3' (complementary sequence); 5’-AGAAGATATTTGGAATAACATGACCAGGATGCA-3’ (one-base mismatch sequence); 5’-AGAAGATAATTGGAATAACATGACCAGGATGCA-3’

(two-bases mismatch sequence); 5’-AGAAGATAATTGGATTAACATGACCAGGATGCA-3’ (three-bases mismatch secquence); 5'-TGCATCGAGGTCATGTTATTCCATATATGTTCT-3' (non-complementary sequence).

Substrate preparation

Five kinds of substrates were prepared for following experiments.

(1) gold substrate (Au): Gold substrate was fabricated with a two-layer structure of 2 nm chromium and 50 nm gold deposited on a glass substrate through a sputtering deposition apparatus in a vacuum. The gold substrate was incubated overnight with a 1 mM mercaptoacetic acid in ethanol, followed by rinsing with ethanol and water. As a result, the carboxylic group will be modified on the surface for the following experiments.

(2) graphene oxide modified glass substrate (GO): Glass substrate was incubated in ethanol with 1% 3-aminopropyltriethoxysilane for 1 h, followed by rinsing. With the addition of 50 μL graphene oxide solution on the substrate, spin-coating was performed at 100 rpm for 10 s and at 3000 rpm for another 40 s. The amino group modified on the substrate will be helpful to acid the adsorption of graphene oxide. Then, the substrate was incubated in DMF with 1 mM 1-Pyrenebutyric acid for 2h, followed by rinsing. Through π–π stacking, 1-Pyrenebutyric acid can be efficiently modified on the graphene oxide surface to expose the carboxylic group for the following modification.

(3) graphene oxide modified gold substrate (Au+GO): A bare gold substrate was fabricated with a two-layer structure of 2 nm chromium and 50 nm gold deposited on a glass substrate through a sputtering deposition apparatus in a vacuum. The gold substrate was incubated overnight with a 1 mM cysteamine in ethanol, followed by rinsing with ethanol and water. With the addition of 50 μL graphene oxide solution on the amino group modified substrate, spin-coating was performed at 100 rpm for 10 s

and at 3000 rpm for another 40 s. Then, the graphene oxide adsorbed substrate was incubated in DMF with 1 mM 1-pyrenebutyric acid for 2h, followed by rinsing, to link the carboxylic group on the surface.

(4) dielectric isolation medium modified gold substrate (Au+isolation): A continuous layer of 2 nm chromium, 50 nm gold, and 30 nm SiO_2 was deposited onto a glass substrate through a sputtering deposition apparatus in a vacuum. The substrate was incubated in ethanol with 1% 3-aminopropyltriethoxysilane for 1 h, followed by rinsing. The substrate was then incubated in DMF with 50 mM glutaric anhydride for 5 h, followed by rinsing, to creat carboxyl-functionalized surface.

(5) FEIMB substrate: A continuous layer of 2 nm chromium, 50 nm gold, and defined thickness of SiO_2 was deposited onto a glass substrate through a sputtering deposition apparatus in a vacuum. The substrate was incubated in ethanol with 1% 3-aminopropyltriethoxysilane for 1 h, followed by rinsing. With the addition of 50 μL graphene oxide solution on the amino group modified substrate, spin-coating was performed at 100 rpm for 10 s and at 3000 rpm for another 40 s. Then, the graphene oxide adsorbed substrate was incubated in DMF with 1 mM 1-pyrenebutyric acid for 2h, followed by rinsing, to link the carboxylic group on the surface for the following modification. As shown in Figure S1, the typical SEM images of the FEIMB substrate indicate graphene oxides can be modified on the substrate with a large coverage when 50 μL graphene oxide solution was added for coating. As shown in Figure S2, AFM images show the morphological changes in preparing FEIMB substrate. As shown in Figure S3, attenuated total reflection Fourier transform infrared spectra prove the modification of graphene oxide on FEIMB substrate.

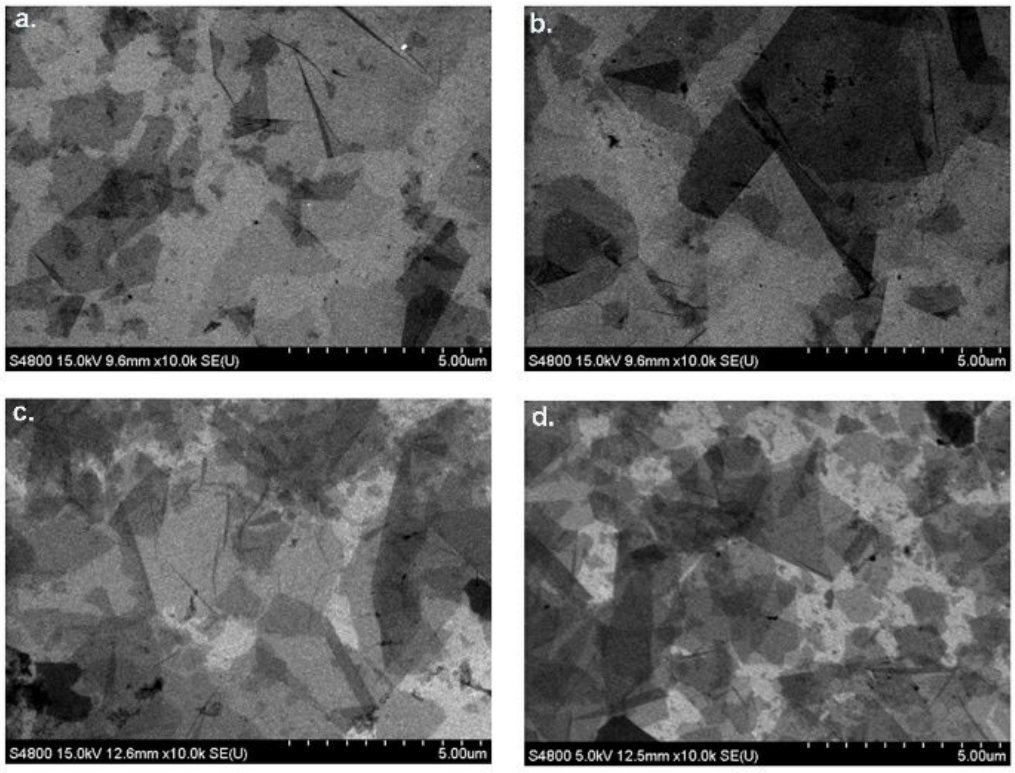

Figure S1. SEM images of the FEIMB substrate fabricated by the addition of graphene oxide with different volumes of 20 μL (a), 35 μL (b), 50 μL (c), and 65 μL (d).

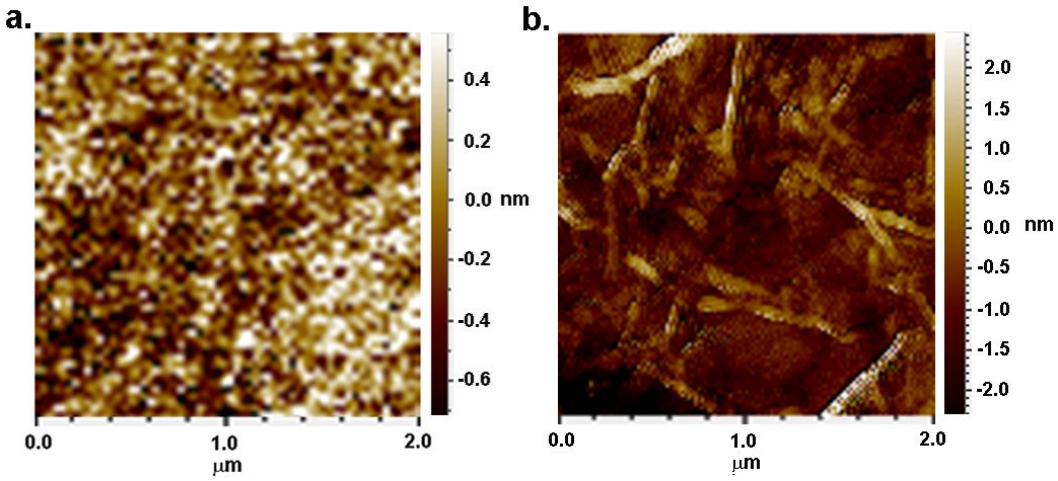

Figure S2. Height AFM images of the FEIMB substrate before (a) and after (b) graphene oxide modification.

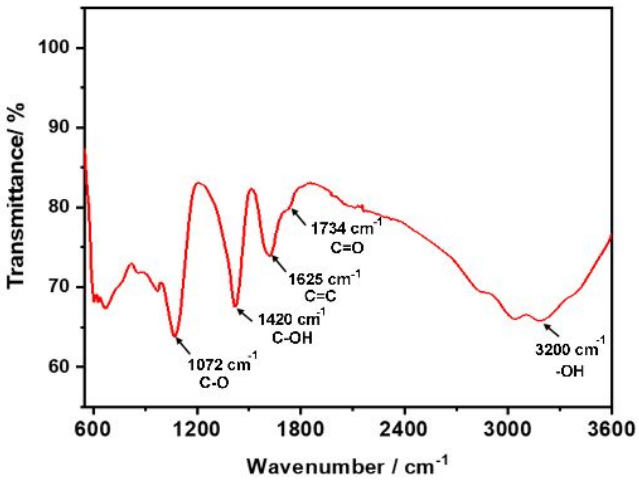

Figure S3. Attenuated total reflection Fourier transform infrared spectra of FEIMB substrate.

Measurement

As shown in Figure S4, SPCE signal was detected with a home-made multifunctional spectrofluorimeter. The sensing chip (indluding Au, Au+GO, Au+isolation, and FEIMB) was attached with index-matching fluid to a semi-cylindrical prism positioned on a precise rotary stage. In the reverse Kretschmann (RK) configuration, the incident light (532 nm laser) was normal to the sample interface. The SPCE signal was collected at a directional angle via the prism. To avoid signal loss absorbed by GO, the detection in GO was measured in the front of the chip. To avoid interference from excitation source, 560 nm long-pass filter was inserted in the spectrometer. During the polarization measurement, p-polarizer and s-polarizer was respectively inserted in the spectrometer.

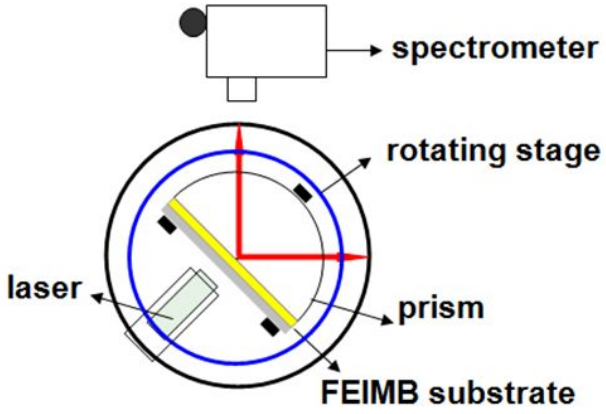

Figure S4. The geometry for directional SPCE measurement in reverse Kretschmann (RK) configuration.

Model experiments to monitor distance changes

The above five kinds of substrate have been carboxy-functionalized to be negative charged. Through the layer-by-layer assembly strategy, polyelectrolyte layers can be fabricated on these substrates. 1 mg/mL PSS and 1.5 mg/mL PAH were dissolved in 1 M NaCl solution respectively. These substrates were immersed in a PAH (positive charged) solution for 20 min, followed by washing, and then immersed again in a PSS (negative charged) solution for 20 min, followed by washing. The assembly was repeated in a layer-by-layer way to create separation layers. 1 μM Rhodamine 6G (positive charged) was finally adsorbed for 30 min, followed by washing. One assembly of PAH and PSS was considered to be 5 nm according to previous reports. Through changing numbers of assembled layers, the distance between substrates and fluorophores can be precisely controlled. Herein, a distance change from 0 to 15 nm was created by separation layers to mimic usual conformational changes for bioswitch. Through varying the thicknesses of SiO_2 layers deposited on the FEIMB substrate, quenching-to-enhancing region can be flexibly modulated. As a result, the optimized FEIMB substrate can be fabricated to adapt the scale match of the desired distance change, achieving a low initial background signal and a high readout signal.

As shown in Figure S5, when polyelectrolyte layers were added to mimic potential conformational changes to separate fluorophores from substrates, greatly enhanced FEIMB signal and quenched initial background can be achieved through screening the modulation layer, which demonstrates that FEIMB should be an efficient approach to reconstruct quenching-to-enhancing region adapted to the required conformation-switching. According to profiles of the modulation layer dependent FEIMB signals responding to varied distances (Figure S5b to 5d), it is suggested that 30 nm dielectric isolation medium can be one good choice to construct relative high-performence devices for general and convenient applications, towards varied distance changes in practice. To maintain the consistency of the comparison, one thickness of 30 nm SiO_2 was chosen for fabricating the dielectric isolation medium in substrate comparison tests under varied distance changes. The response sensitivity was evaluated by signal-to-background ratio (S/B), which was defined as $S/B = (F_{after} - F_{blank})/(F_{before} - F_{blank})$, where F_{before} and F_{after} indicated the fluorescence signals before and after sensing processes, and F_{blank} was the detected blank signal from the equipment. In the model experiments, F_{before} and F_{after} corresponded to the situations without and with polyelectrolyte layers respectively.

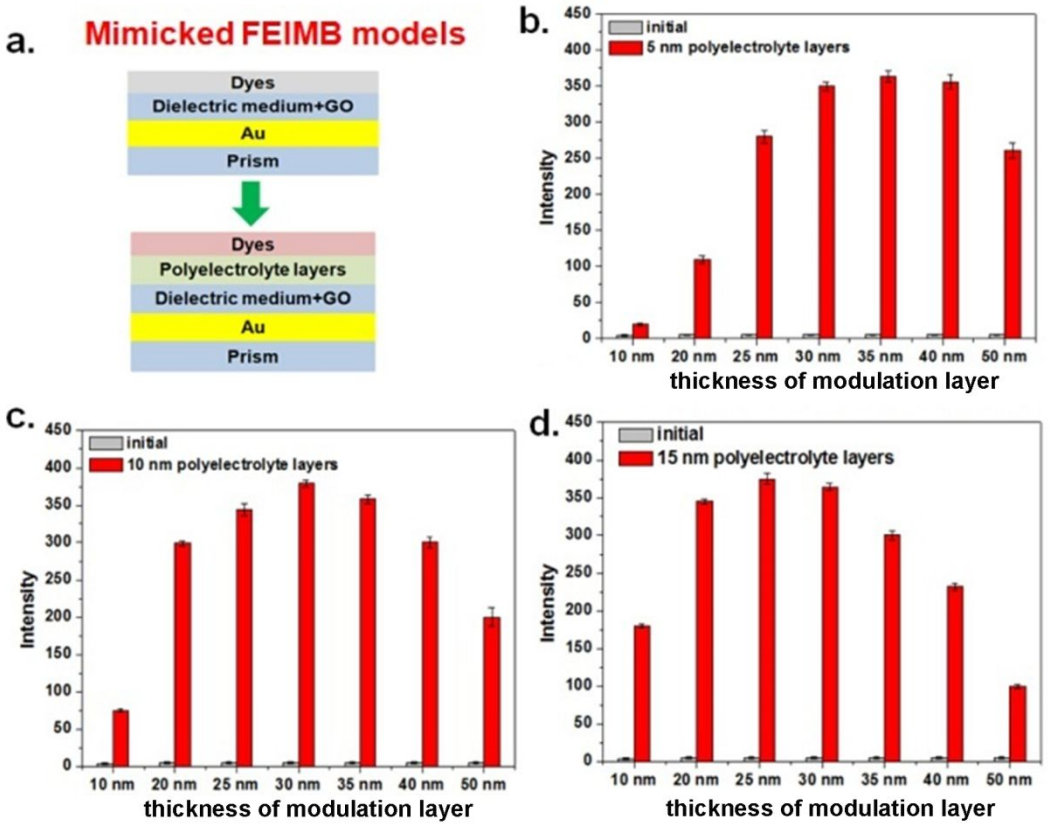

Figure S5. (a) Sketch of experiments to mimic conformation-induced distance changes in FEIMB. (b-d) FEIMB signals changing with varied thicknesses of the modulation layer when mimicked conformational changes were 5 nm (a), 10 nm (c), and 15 nm (d).

Protein detection

GO, Au+GO, and FEIMB (30 nm SiO_2) substrates were incubated in solution with 0.1 M MES, 10 mM EDC and 10 mM NHS for 30 min to activate interfacial carboxyl. After rinsing, 1 μM amino functionalized aptamer sequence was added with an incubation for 1 h (as shown in Figure S6, 1 μM could be the appropriate probe concentration). After rinsing, 50 mM glycine solution was used to quench the redundant activated sites for an incubation time of 30 min. After rinsing, aptamer linked substrates can be used for protein detection with the incubation time of 15 min. The limit of detection was calculated according to signal equal to the background

signal plus three times the standard deviation of the control measurements.

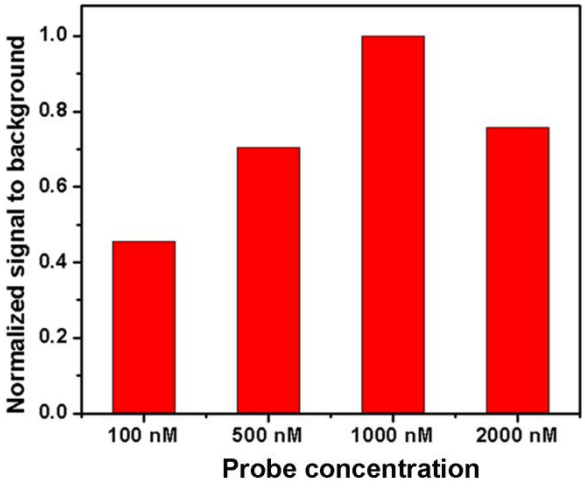

Figure S6. Effects of the thrombin aptamer probe concentrations on normalized signal to background (under100 nM thrombin as the target).

The simulated reflectivity curves in Figure S7 were drawn based on Fresnel calculations at wavelength of 585 nm. The p-polarized fitting curve with the maximum absorption at 53 degree was achieved when 4.5 nm thickness was used as the simulation parameter, which was consistent to the experimental results with the directional SPCE signal at 53 degree and ～4.5 nm distance changes can thus be expected after sensing thrombin. The p-polarized emission in SPCE was confirmed as shown in Figure S8. FEIMB was operated with positive responding to thrombin (Figure S9). To test the specificity of FEIMB, artificial matrix including 10-fold excess of other proteins and fetal calf serum were separately added, and the specificity was guaranteed (Figure S10). Through 2 min incubation of 6 M guanidine hydrochloride solution to break the interaction between thrombin and aptamer, FEIMB was regenerated and the cyclical measurements were realized (Figure S11).

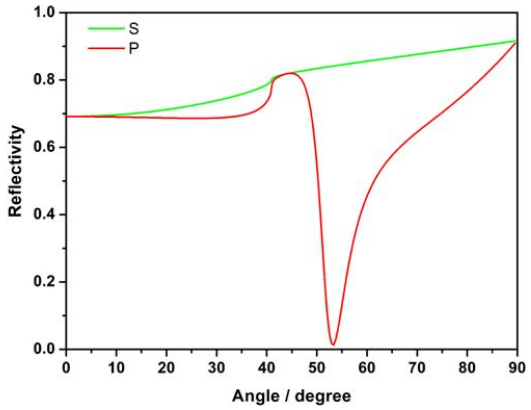

Figure S7. Fresnel theoretical reflectivity to simulate FEIMB sensing.

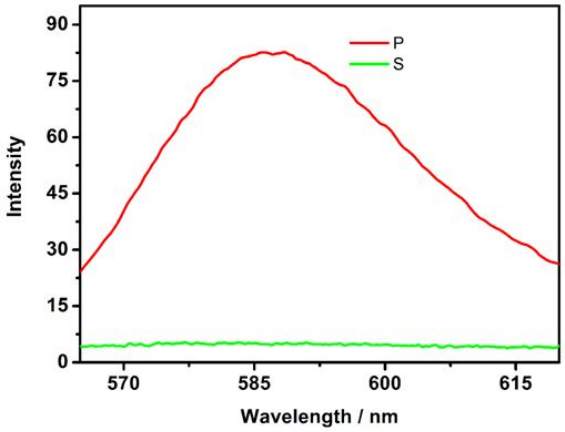

Figure S8. Polarized emission spectra in FEIMB with the insertion of the p polarized and the s polarized polarizers respectively in the spectrometer.

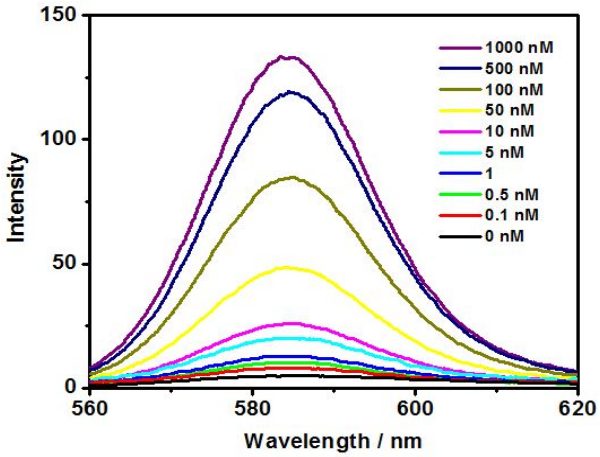

Figure S9. SPCE spectra in FEIMB responding to a series of thrombin concentrations.

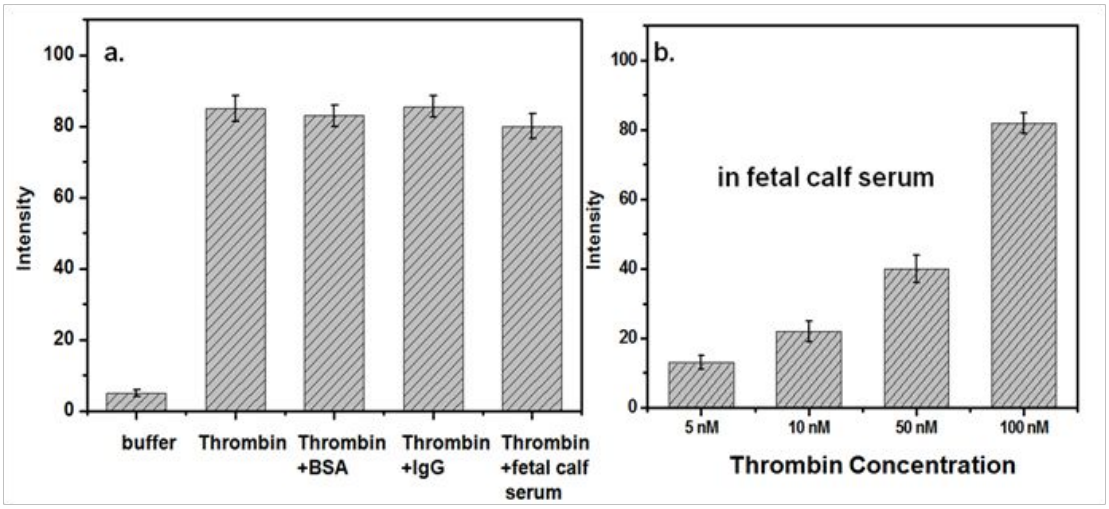

Figure S10. (a) FEIMB signals responding to PBS buffer, 100 nM thrombin, 1 μM BSA+100 nM thrombin, 1 μM IgG+100 nM thrombin, and 15% fetal calf serum+100 nM thrombin. (b) Thrombin concentration dependent FEIMB signal in the medium of 15% fetal calf serum.

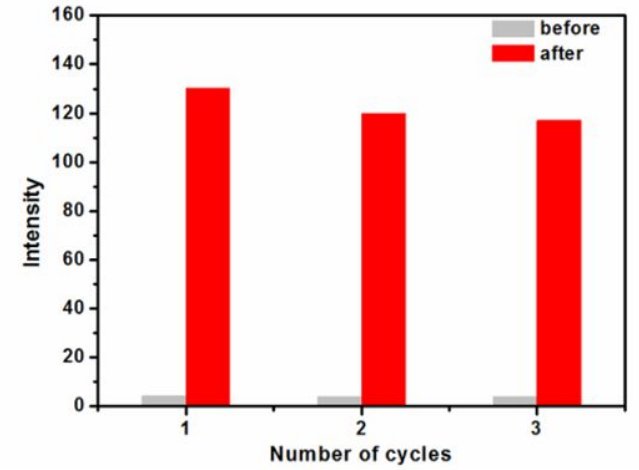

Figure S11. The regeneration of FEIMB sensing in protein detection.

DNA detection

FEIMB substrates with the dielectric isolation medium from 10 nm to 50 nm were used in DNA lengths tests. FEIMB substrates with 30 nm SiO_2 were used in HIV biosensing. GO, Au+GO, and FEIMB substrates were incubated in solution with 0.1 M MES, 10 mM EDC and 10 mM NHS for 30 min to activate interfacial carboxyl. After rinsing, 1 μM amino functionalized DNA probe was added with the incubation

for 1 h (as shown in Figure S12, 1 μM could be the appropriate probe concentration). DNA probes with varied lengths were used to fabricate substrates. After rinsing, 50 mM glycine solution was used to quench the redundant activated sites for an incubation time of 30 min. After rinsing, tested DNA sequences were incubated for 1 h before optical measurements. The regeneration of FEIMB in DNA assays was operated through the incubation in 90 ℃ water for 2 min.

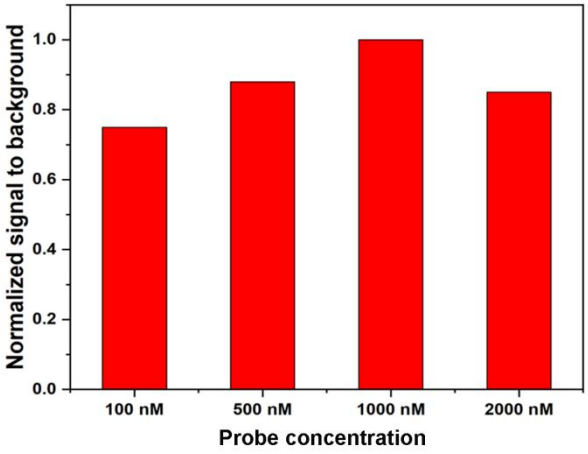

Figure S12. Effects of HIV probe concentrations on normalized signal to background ratio (under 100 nM matched DNA as the target).

The sensing ability was tested to detect DNA with different lengths of 8 bases, 16 bases, 24 bases, and 48 bases. As shown in Figure S13, FEIMB substrates with varied modulation layers were used to screen the optimized devices towards the defined DNA lengths. Experimental results demonstrate that it is an efficient approach to obtain high signal-to-background DNA sensors through appropriate FEIMB designs. In FEIMB based HIV detection, the simulated reflectivity curves in Figure S14 were drawn according to Fresnel calculations at wavelength of 585 nm. The p-polarized fitting curve with the maximum absorption at 54.5 degree was achieved when 7.5 nm thickness was used as the simulation parameter, which indicates DNA hybridization induced conformational change would be ～ 7.5 nm in this case. The p-polarized emission in SPCE was confirmed as shown in Figure S15. FEIMB was operated with positive responding to the complementary sequence (Figure S16). And FEIMB

presented the capacity to distinguish mismatched sequence (Figure S17). The positive response of increased signal with increasing target DNA concentrations in the presence of matrixes supported the potential applications of this sensor in practice (Figure S18). After three regeneration cycles, no less than 90% signal responding can be observed, holding the promise in practical applications (Figure S19).

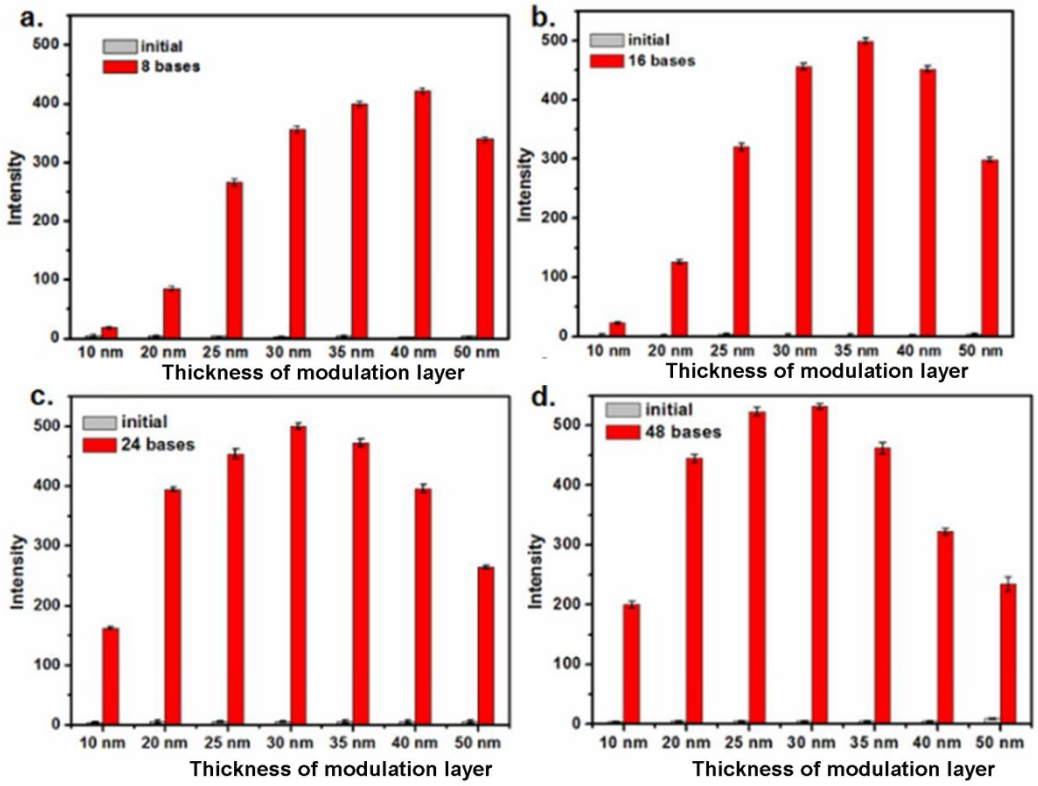

Figure S13. (a-d) FEIMB signals changing with varied thicknesses of the modulation layer when DNA hybridization occurred with lengths of 8 bases (a), 16 bases (b), 24 bases (c), and 48 bases (d).

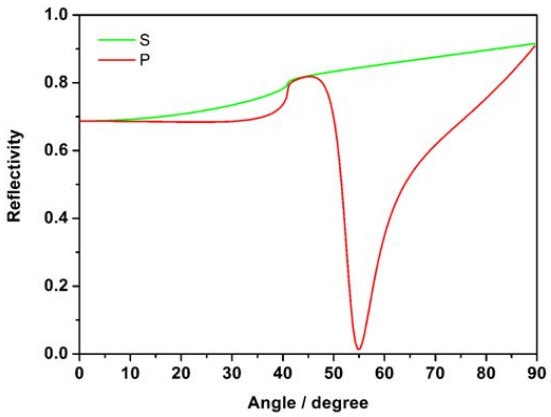

Figure S14. Fresnel theoretical reflectivity to simulate FEIMB sensing.

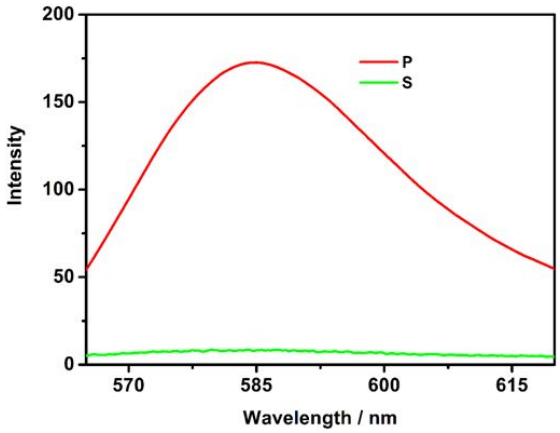

Figure S15. Polarized emission spectra in FEIMB with the insertion of the p polarized and the s polarized polarizers respectively in the spectrometer.

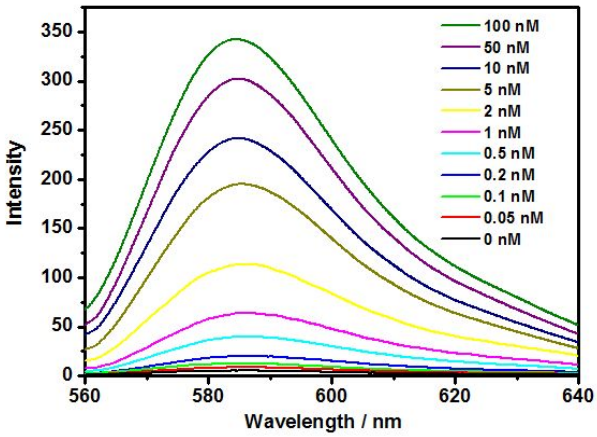

Figure S16. SPCE spectra in FEIMB responding to a series of DNA target concentrations.

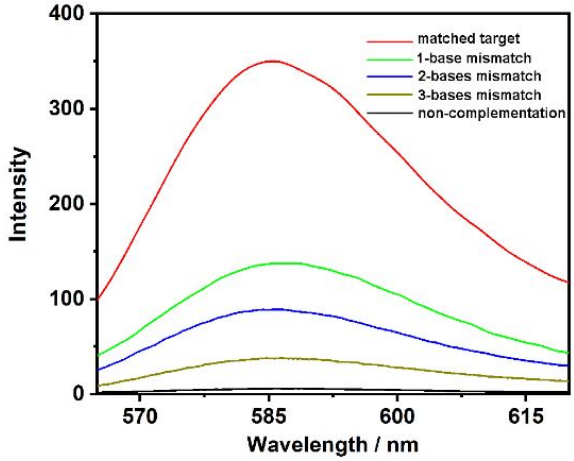

Figure S17. SPCE spectra in FEIMB after sensing with perfectly matched DNA target (red curve), 1-base mismatched sequence (green curve), 2-bases mismatched sequence (blue curve), 3-bases mismatched sequence (yellow curve), and non-complementary sequence (black curve).

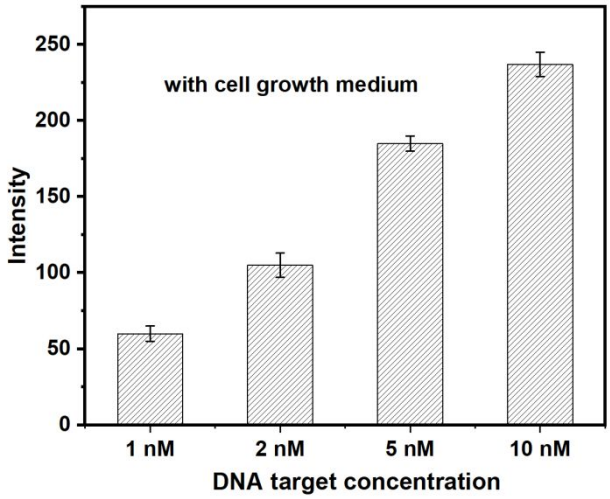

Figure S18. DNA target concentration dependent FEIMB signal in the presence of cell growth medium.

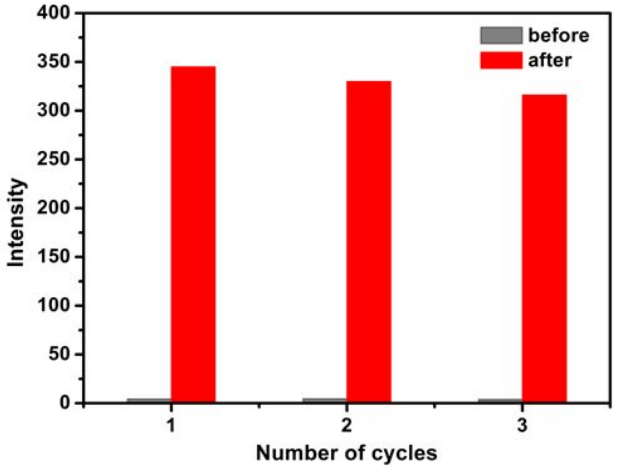

Figure S19. The regeneration of FEIMB sensing in DNA detection.

References

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