Electronic Supplementary Information (ESI) for

An anti-fouling aptasensor for detection of thrombin by dual polarization interferometry

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DPI instrumentation and Principle: DPI is mainly composed of a helium-neon laser emitting light at 632.8 nm, a controller to switch plane polarized light (transverse magnetic (TM) and transverse electric (TE)), an optical sensing waveguide of multiple layers, an array photodiode and a fluidics system. The sensing waveguide mainly consists of sensing layer and reference layer separated by a central glass clad region. The fluidic system consists of a Rheodyne HPLC injector Valve and an external pump (Harvard Apparatus, PHD2000) to provide a controlled continuous fluid flow over channels on the chip surface. The polarized light coupled in at one end of the waveguide are combined with each other at the other end of the waveguide, forming Young's Interference fringes in the far-field. Each slight change in the fluid will affect the interface of the sensing layer, induce the phase change of the sensing light path output, thereby changing the positions of Young's Interference fringes. The two polarizations TE and TM give two independent phase shift response, after the analysis of which, real-time thickness, density and mass values of the surface layers will be obtained.

Regents and Chemicals: Thrombin from human plasma, lysozyme, casein, bovine serum albumin (BSA), streptavidin (SA) and methoxypolyethylene glycol amine (NH_2-PEG, Mw. 750) were purchased from Sigma -Aldrich (St Louis, MO, USA). Glutaraldehyde (GA, 25% v/v) aqueous solution was purchased from Acros Organics. All of the modified oligonucleotides were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). Before use, oligonucleotides were dissolved in ultrapure water as stock solutions stored in refrigerator at 4 °C, and the concentrations were quantified by measuring the UV absorption at 260 nm. Other regents were of analytical grade and used without further purification or treatment. Ultrapure water (Milli-Q synthesis, Millipore Inc., Bedford, MA) was used throughout. 10 mM pH 7.4 PBS buffer solution (10 mM NaH_2PO_4-Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl) was prepared for functional assembly of the chip surface. 10 mM pH 7.4 HEPES buffer solution (10 mM HEPES-NaOH, 120 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$) was prepared for studying interactions between thrombin and oligonucleotides. All the buffer solutions were filtered and degassed before use.

Silanization of DPI chip: Prior to silanization, unmodified silicon oxynitride

AnaChip (FB 80, Farfield Scientific Ltd.) was first pretreated with Piranha solution (a 7:3 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) for 3 hours. Then, rinsed the chip in ultrapure water under sonication for six times (6 min each time), followed by drying it under a stream of nitrogen to get rid of water from the surface. Placed the chip in an oven at 110 °C for 1 h, then the chip was ready for silanization. Soaked the chip in freshly prepared 2% 3-APTES in toluene solution for 2 h. Subsequently, rinsed the chip in toluene for 3 min and dried it under a stream of nitrogen. Finally, cured the chip in an oven at 110 °C for 1 h prior to storage[1].

General procedure for DPI experiment: DPI measurements were performed in real time utilizing DPI instrument (AnaLight Bio200, Farfield Group Ltd., Crewe, U.K.). All experiments were performed at 20 ± 0.002 °C. After the silanized chip was loaded into the instrument, 10 mM PBS buffer as the running buffer solution was passed over the chip at a flow rate of 50 μL/min. After the baseline was stable, an ethanol/water (80%, v/v) solution and ultrapure water of known refractive indexes were sequentially injected to calibrate the chip and the running buffer, through which thickness and refractive index of the top waveguide layer and the refractive index of the running buffer were obtained and applied for the following experiment data calculation.

After calibration, the flow rate was set at 20 μL/min. GA solution (0.4%, v/v), SA solution (0.5 mg/mL), BSA solution (5 mg/mL) and NH_2-PEG solution (3 mg/mL) were sequentially injected for 10 min. After each injection, the flow was returned to the running buffer to stabilize the baseline. Then, changed the running buffer to 10 mM HEPES buffer. When the baseline was stable, 2 μM thrombin-binding aptamer or control DNA was injected for 10 min. Then, thrombin solutions of different concentrations ranging from 1 nM to 500 nM were injected independently for 10 min. Between each injection of thrombin, 20 μL of 1.2 M NaCl solution was injected to regenerate the chip surface.

References

1. C. M. Halliwell and A. E. G. Cass, *Analytical Chemistry*, 2001, 73, 2476-2483.

Table S1 All oligonucleotide sequences used in DPI experiments.

Name	Sequence
EG-T_5-TBA	5'-biotin-$(CH_2CH_2O)_6$-TTT TT GGT TGG TGT GGT TGG-3'
EG-TBA	5'-biotin-$(CH_2CH_2O)_6$- GGT TGG TGT GGT TGG-3'
TBA	5'-biotin- GGT TGG TGT GGT TGG-3'
T_5-TBA	5'-biotin- TTT TT GGT TGG TGT GGT TGG-3'
T_{10}-TBA	5'-biotin- TTT TTT TTT T GGT TGG TGT GGT TGG-3'
EG-T_5-RAN	5'-biotin-$(CH_2CH_2O)_6$-TTT TT GGT GGT TGT TGT GGT-3'
EG-RAN	5'-biotin-$(CH_2CH_2O)_6$- GGT GGT TGT TGT GGT-3'
T_5-RAN	5'-biotin- TTT TT GGT GGT TGT TGT GGT-3'
T_{10}-RAN	5'-biotin- TTT TTT TTT T GGT GGT TGT TGT GGT-3'

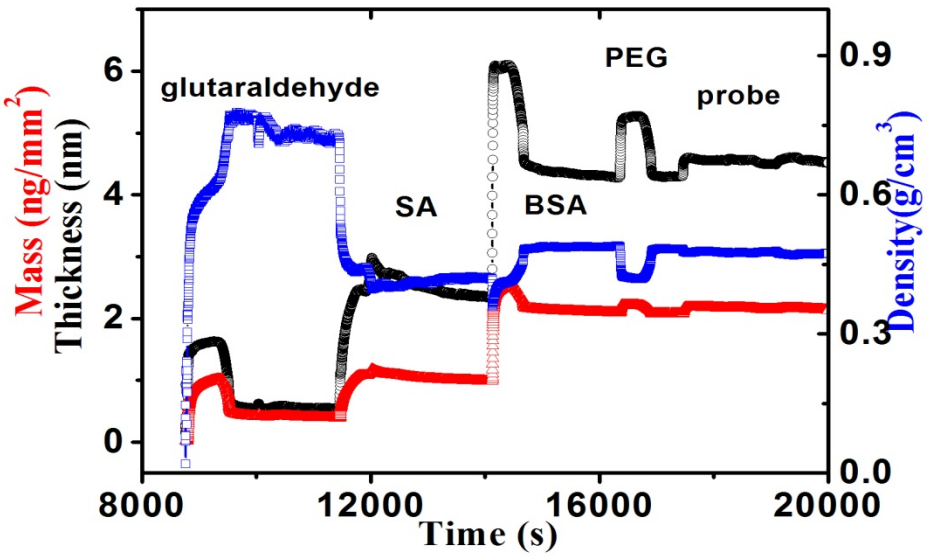

Fig. S1 DPI-based real-time measurements of mass (red triangles), thickness (black circles) and density (blue squares) values for the whole immobilization process.

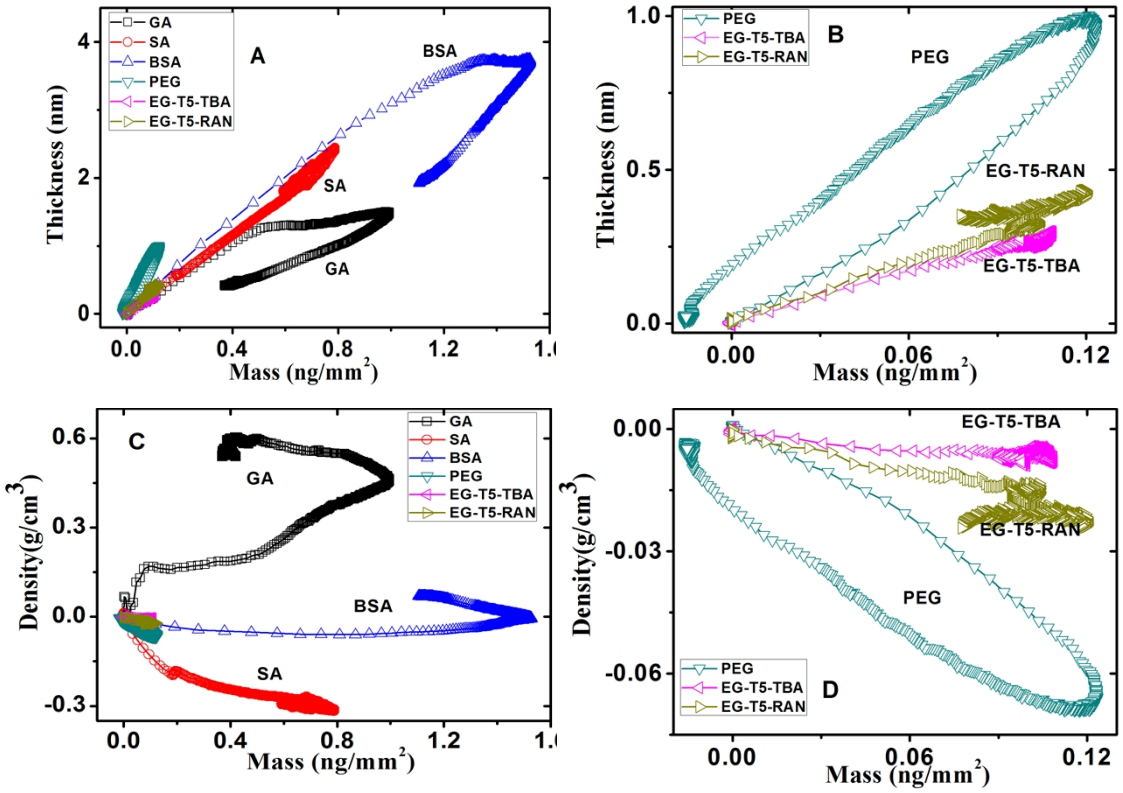

Fig. S2 Changes of layer thickness (A, B) and density (C, D) values as functions of the respective mass loading during the entire process of each immobilization from Fig. S1.

Table S2 Layer structure for the immobilized layer (Mean ± SD, n =3).

Layer material	Mass (ng/mm^2)	Thickness (nm)	Density (g/cm^3)
0.4% GA	0.412±0.028	0.559± 0.014	0.737± 0.032
0.5 mg/mL SA	1.022±0.051[a]	2.302± 0.075[a]	0.444± 0.036[a]
5 mg/mL BSA	2.108± 0.026[a]	4.158± 0.177[a]	0.508± 0.028[a]
3 mg/mL NH_2-PEG	2.098± 0.034 [a]	4.187± 0.157 [a]	0.502± 0.026 [a]
2 μM EG-T_5-TBA	2.153± 0.033[a]	4.495± 0.085[a]	0.494± 0.016[a]
2 μM EG-T_5-RAN	2.150± 0.053[a]	4.574±0.064[a]	0.479± 0.021 [a]

[a] Mass, thickness and density values of the whole layer.

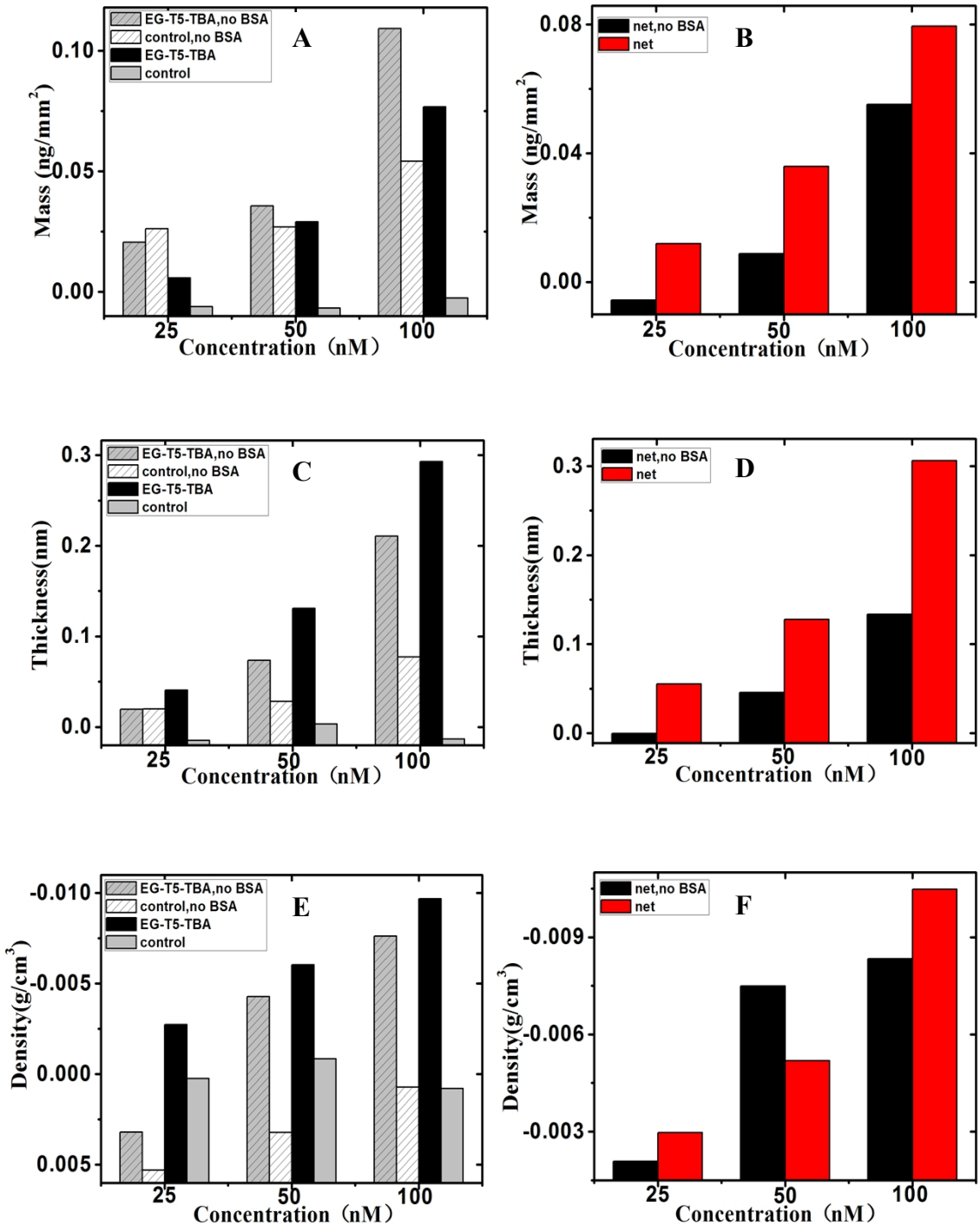

Fig. S3 Comparison of Mass (A, B), thickness (C, D) and density (E, F) value changes of layers blocked with PEG or with both BSA and PEG upon the addition of different concentrations of 200 μL thrombin. The concentrations of thrombin are: 25 nM, 50 nM and 100 nM.

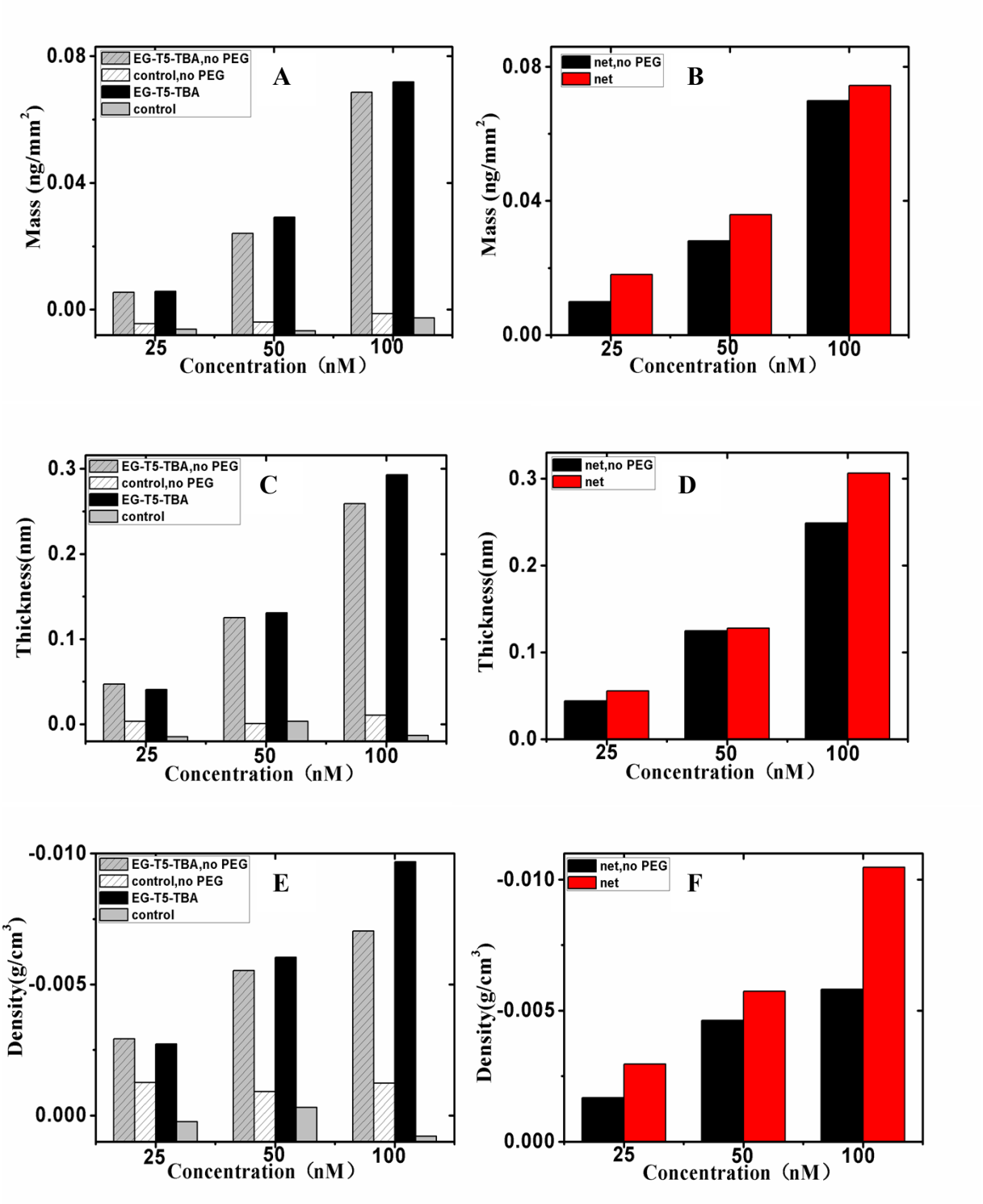

Fig. S4 Comparison of Mass (A, B), thickness (C, D) and density (E, F) value changes of layers blocked with BSA or with both BSA and PEG upon the addition of different concentrations of 200 μL thrombin. The concentrations of thrombin are: 25 nM, 50 nM and 100 nM.

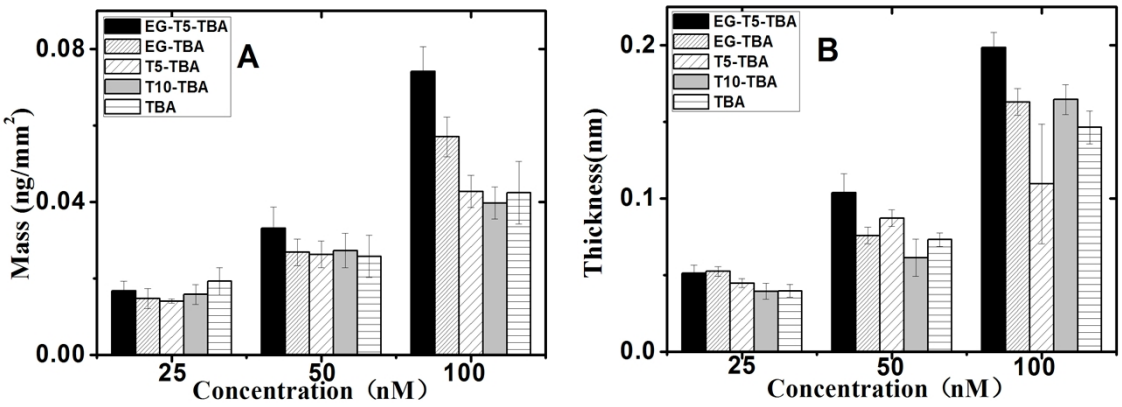

Fig. S5 Comparison of Mass (A) and thickness (B) value changes of layers modified with different designed thrombin-binding aptamers upon the addition of different concentrations of 200 μL thrombin. The concentrations of thrombin are: 25 nM, 50 nM and 100 nM.

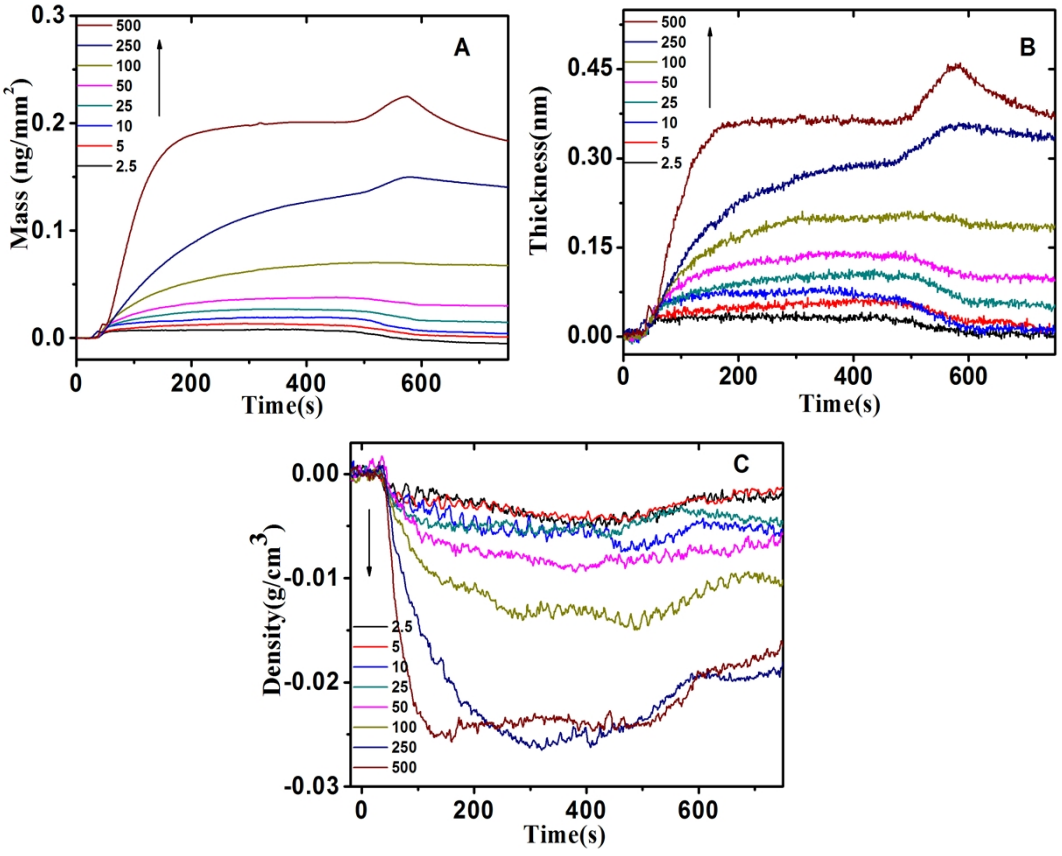

Fig. S6 Real-time mass (A), thickness (B) and density (C) value changes of the EG-T_5-TBA layer upon thrombin injection. The concentrations of thrombin are: 2.5, 5, 10, 25, 50, 100, 250, 500 nM.The arrows show increasing thrombin concentration.

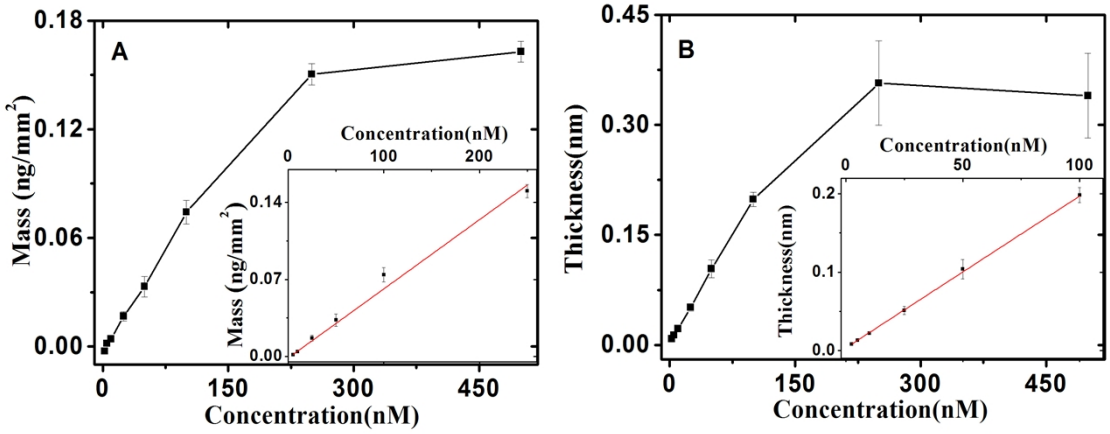

Fig. S7 Mass (A) and thickness (B) value changes of the EG-T_5-TBA layer after the addition of different concentrations of thrombin ranging from 1 nM to 500 nM. The error bars represent the standard deviation of three measurements.

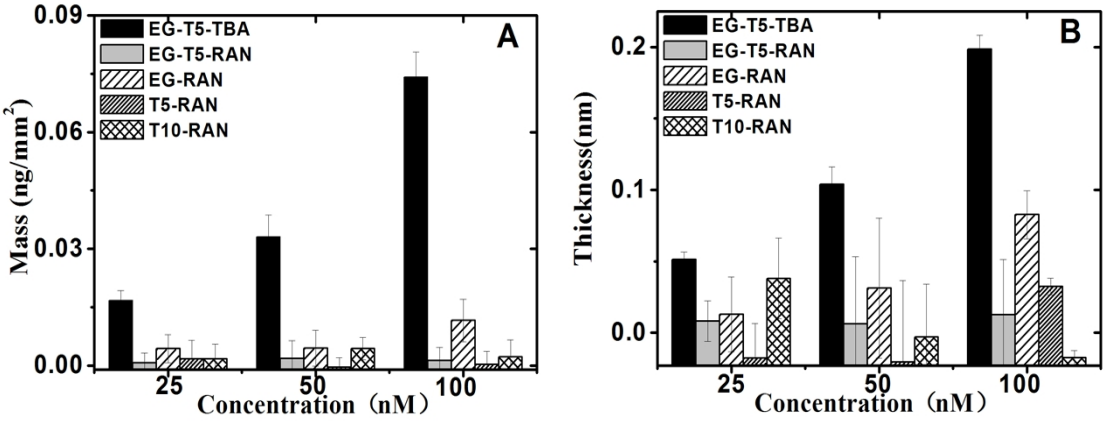

Fig. S8 Comparison of Mass (A) and thickness (B) value changes of layers modified with EG-T_5-TBA and different RANs upon the addition of different concentrations of 200 μL thrombin. The concentrations of RANs injected are 2 μM, the concentrations of thrombin are: 25 nM, 50 nM and 100 nM.

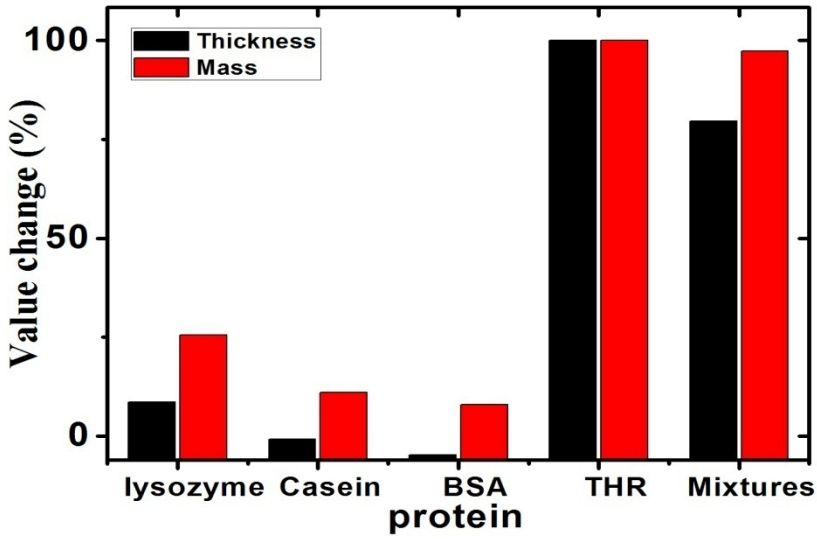

Fig. S9 Selectivity of the aptasensor for thrombin over other proteins: lysozyme, casein and BSA. The concentration of the other proteins is 0.01 mg/mL and the concentration of thrombin is 0.003 mg/mL.