

Electronic Supplementary Information

An Affinity Capture Involved Enzymatic Assay for Thrombin by Using Peptide Aptamer as Affinity Ligands on Magnetic Beads

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Chemical and materials

Peptides were synthesized by Sangon Biotech (Shanghai, China). The thrombin-binding peptide had the following sequence, biotin-Ahx-MGMGT CFFDR YDSAR DPGRL LSGSS MSGS-COOH, and a 6-carbon spacer (Ahx, aminohexanoic acid) was introduced between biotin and the peptide. The control peptide had the following sequence, biotin-Ahx-MGMGT CVRIA ASRIS TNNKY FSGSS MSGS-COOH. The biotinylated 29-mer DNA aptamer against thrombin was also synthesized by Sangon Biotech (Shanghai, China), having the following sequence, 5'-biotin-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3'. Human alpha thrombin and human plasma sample were purchased from Haematologic Technologies Inc. Trypsin (from bovine pancreas), bovine serum albumin (BSA), human serum albumin (HSA), human immunoglobulin G (IgG), and hemoglobin from bovine blood were ordered from Sigma. Chymotrypsin and porcine elastase were purchased from Ruibio. Proteinase K was obtained from Merck. The fluorogenic substrate of thrombin, *N-p*-tosyl-Gly-Pro-Arg-7-amido-4-methylcoumarin hydrochloride, and the chromogenic substrate of thrombin, *N-p*-tosyl-Gly-Pro-Arg-p-nitroanilide acetate were purchased from Sigma. Streptavidin coated magnetic beads (Dynabeads MyOne™ Streptavidin C1) were purchased from Invitrogen Dynal. The magnetic beads had a

diameter around 1 μm .

Preparation of peptide modified magnetic beads

50 μL of peptide at 14 μM was mixed with 50 μL of streptavidin coated magnetic beads (10 mg/mL), and incubated at room temperature for one hour. Then, the magnetic beads were washed with 100 μL of PBS solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , 2 mM KH_2PO_4 , pH 7.5) containing 0.1 % Tween 20 three times. Finally, the magnetic beads were dispersed in 100 μL of PBS solution (pH 7.5) containing 0.1% Tween 20 and 0.1% NaN_3 . The amount of the peptide molecule attached on the magnetic beads was estimated to be about 400 pmole peptide on 1 mg of magnetic beads according to the protocol provided by the company which produced the streptavidin coated magnetic beads. 1 mg of magnetic beads corresponded to about 8.5×10^9 beads. It was estimated that about 2.8×10^4 peptide molecules were immobilized on each magnetic bead. The peptide modified magnetic beads were stored at 4 $^\circ\text{C}$ prior to use. To compare with the performance of DNA aptamer-modified magnetic beads, 29-mer DNA aptamer modified magnetic beads were prepared by following the same procedure described above.

Assay procedure

Thrombin at varying concentrations in 100 μL of binding buffer solution

(137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, 1 mM MgCl₂, 1 mg/mL BSA, pH 7.5) was mixed with 1 μL of peptide-modified magnetic beads, and the mixture was incubated at room temperature for 30 min. Then, the magnetic beads were separated from solution by magnetic separator. The magnetic beads were washed with washing solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, 1 mM MgCl₂, 1 mM MgCl₂, 0.1% Tween 20, pH 7.5) three times. After that, the magnetic beads were redispersed in 20 μL of reaction solution (50 mM Tris-HCl, 150 mM NaCl, pH 8.5) containing 132 μM fluorogenic substrate *N-p*-tosyl-Gly-Pro-Arg-7-amido-4-methylcoumarin. After 2-h incubation at 37 °C, the reaction solution was separated from the magnetic beads, and added into 80 μL of 2% acetic acid solution. Finally, the fluorescence of the collected solution was recorded by a plate reader (Varioskan Flash, Thermo Fisher Scientific, Inc.) with emission at 440 nm and excitation at 370 nm, and the excitation bandwidth was 5 nm in the measurement. When 29-mer DNA aptamer modified magnetic beads were used instead for comparison, the same assay procedure was applied.

For the assay using the chromogenic substrate, after 100 μL of thrombin at varying concentrations was captured by peptide modified magnetic beads, the magnetic beads were washed and redispersed into 20 μL of reaction solution (50 mM Tris-HCl, 150 mM NaCl, pH 8.5) containing 373 μM chromogenic substrate. After 2-h incubation at 37 °C,

the reaction solution was separated from the magnetic beads, and added into 80 μL of 2% acetic acid solution. Finally, an absorbance spectrometer (HITACHI U3010) was used to measure the absorbance at 405 nm of the collected solution with a 50- μL quartz cuvette.

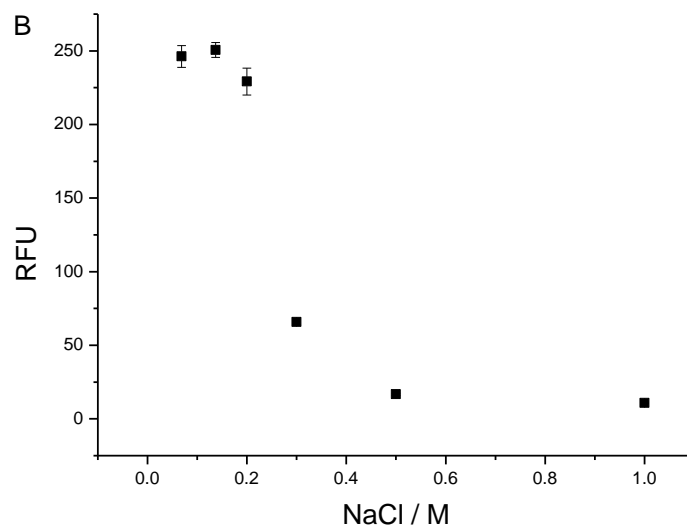
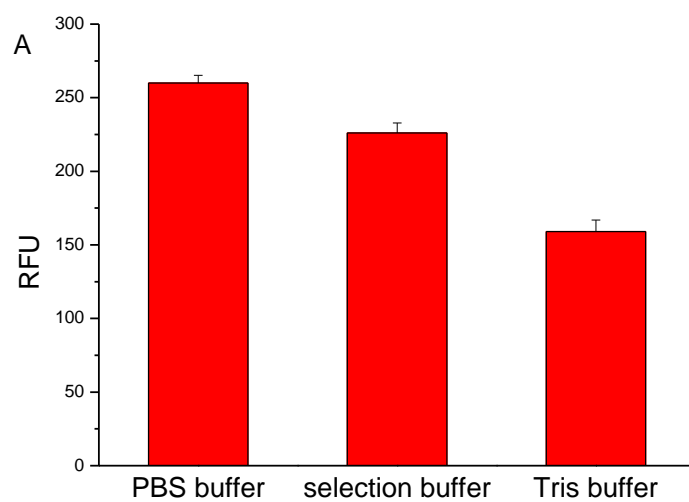


Fig. S1 (A) The effect of binding buffer solutions on the affinity capture of thrombin by peptide aptamer modified magnetic beads in the assay. The tested binding buffer solutions included PBS buffer solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, 1 mM MgCl₂, pH 7.5), selection buffer solution (20 mM HEPES, 140 mM NaCl, 4 mM MgCl₂, 5 mM KCl, 2 mM glutathione, 1 mM glutathione disulfide, pH 7.5), and Tris buffer solution (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 1 mM MgCl₂, pH 7.5). (B) The effect of the concentration of total NaCl in the PBS buffer solution on the affinity capture of thrombin by peptide modified magnetic beads. Thrombin at 25 pM in 100 μL of binding buffer solution containing varying concentrations of NaCl was analyzed.

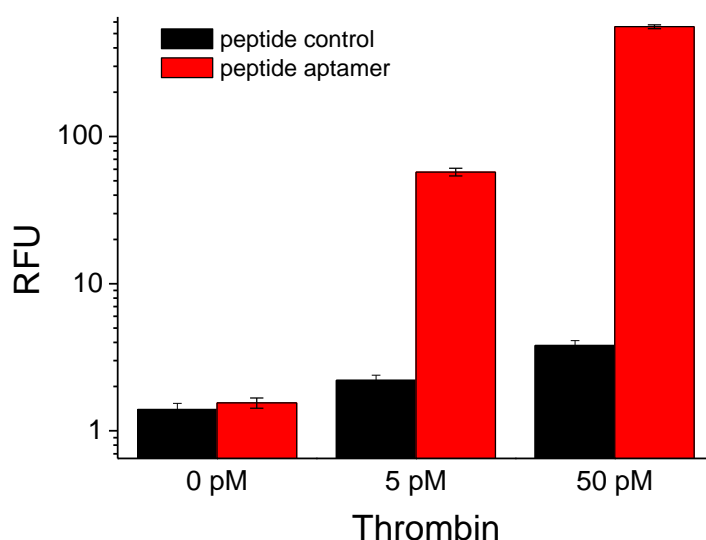


Fig. S2 Test of sequence specificity of the peptide aptamer for thrombin.

Thrombin was analyzed by using peptide aptamer modified magnetic beads or peptide control modified magnetic beads. 100 μL of sample was detected and 2-h enzyme reaction was applied.

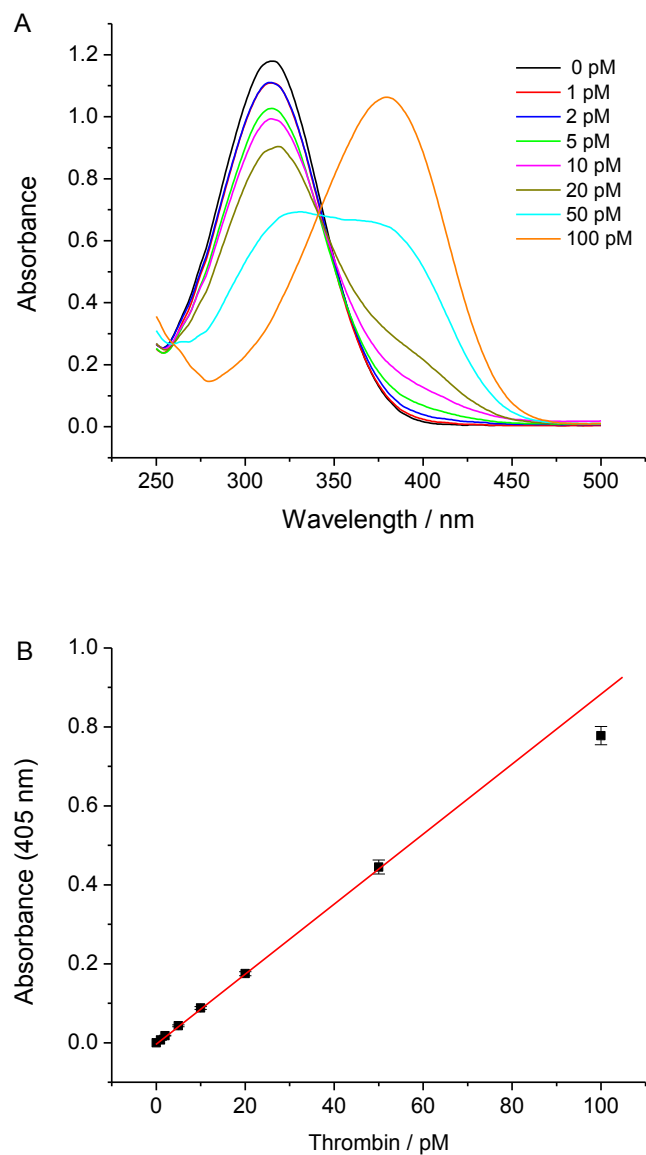


Fig. S3 Detection of thrombin using peptide modified magnetic beads and chromogenic substrate. (A) The recorded absorbance spectra of the generated products corresponding to varying concentrations of thrombin ranging from 0 to 100 pM. (B) The relationship between the net increase

of absorbance at 405 nm over the blank and the concentrations of thrombin. 100 μ L of sample was detected and 2-h enzyme reaction was applied.