Electronic Supplementary Information

Visual Detection of Thrombin Using Strip Biosensor through Aptamer-cleavage Reaction with Enzyme Catalytic Amplification

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References.

1. Preparation of gold nanoparticles (AuNPs)

AuNPs with average diameter 15 nm±3.5 nm were prepared using the citrate reduction method according to the reported [1,2]. All glasswares used in this study were thoroughly cleaned in aqua regia (three parts HCl and one part HNO_3), rinsed in doubly distilled water, and oven dried prior to use. 2.7 mL of 1% trisodium citrate was put in a 250 mL, round-bottom flask, containing 100 mL of 0.01% boiled $HAuCl_4$ aqueous solution. One minute later, the solution turned deep wine-red. Boiling was pursued for an additional 10 min; then the colloid solution was stirred for another 15 min without heating any more. The resulting AuNPs solution was stored in amber laboratory bottles at 4 ℃ and characterized by an absorption maximum at 525 nm. We condensed the AuNPs to different concentrated solutions (data not shown) through the centrifugal and chose the 5-fold AuNPs solution to prepare the DNA1-AuNPs-apatmer, DNA2-AuNPs-HRP and DNA2-AuNPs conjugates.

2. Preparation of streptavidin-biotinylated cDNA1 (cDNA1-SA) conjugates

The streptavidin-biotinylated cDNA1 (cDNA1-SA) conjugates were obtained using the previously reported method [3,4]. Briefly, five hundred microlitre of 2 mg/ml streptavidin was mixed with 10 μM biotinylated cDNA1. The mixture was incubated for 2 h at room temperature. After adding 400 μL PBS (pH=7, 0.01M) into the mixture, the solution was centrifuged in dialysis tube for 10 min at 12000 rpm under room temperature, then discarded the supernatant. The above step was repeated for three times. The remaining solution in filter was diluted to 600 μL with PBS.

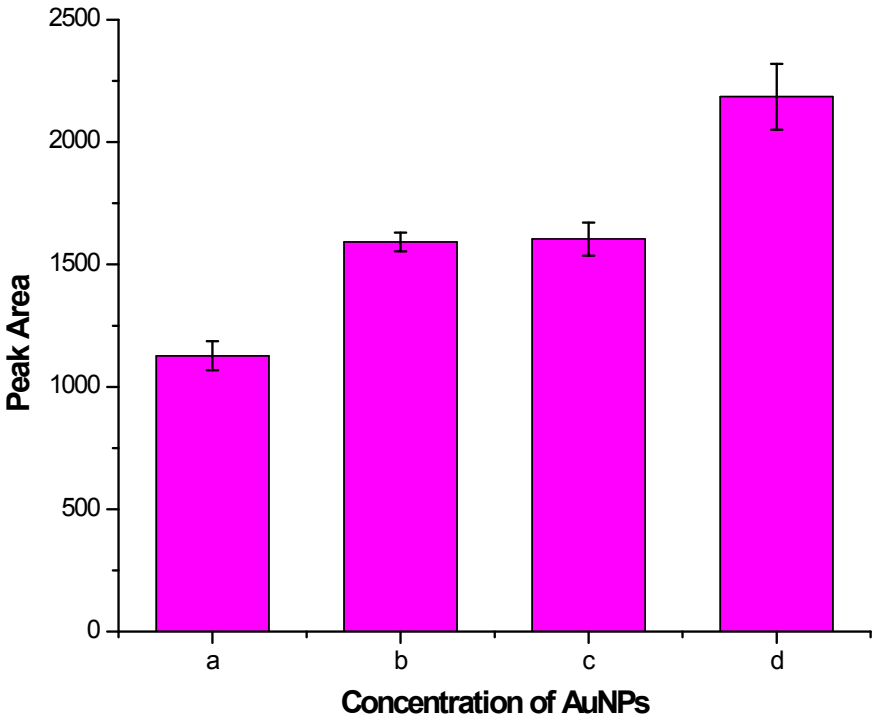


Fig. S1. Plots of the peak areas of red bands on the test zone vs different concentration of AuNPs for 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.

(a:AuNPs-DNA2-HRP: unconcentration AuNPs-DNA1-apatmer: unconcentration; b:AuNPs-DNA2-HRP: unconcentration AuNPs-DNA1-apatmer: five times concentration; c:AuNPs-DNA2-HRP: five times concentration AuNPs-DNA1-apatmer: unconcentration; d:AuNPs-DNA2-HRP: five times concentration AuNPs-DNA1-apatmer: five times concentration).

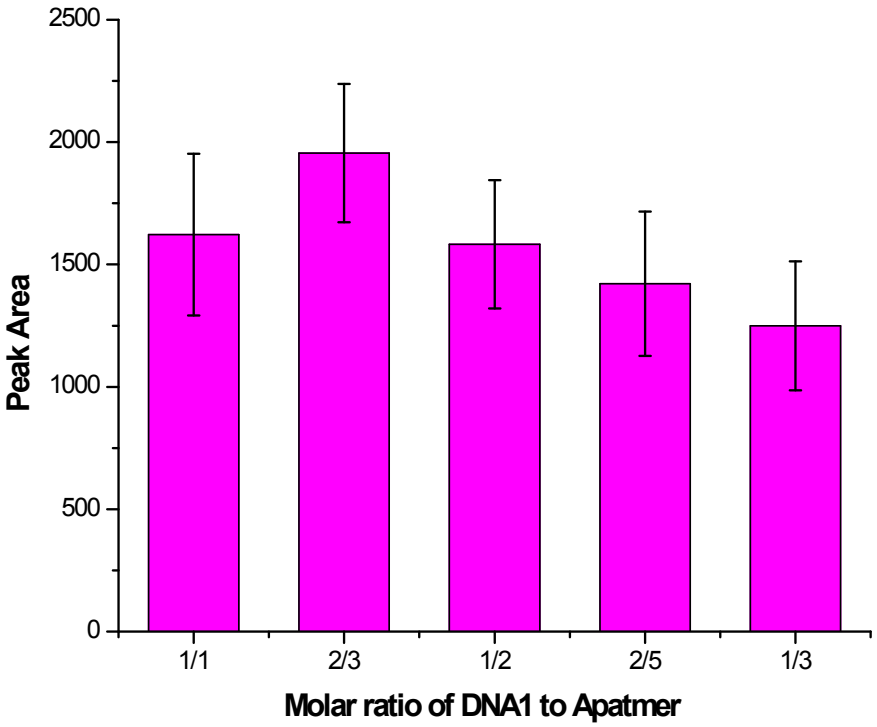


Fig. S2. Effect of different molar ratios of DNA1 to apatmer (1/1, 2/3, 1/2, 2/5, and 1/3) in the DNA1-AuNPs-apatmer compounds. The error bars represent the standard deviation of ten independent measurements.

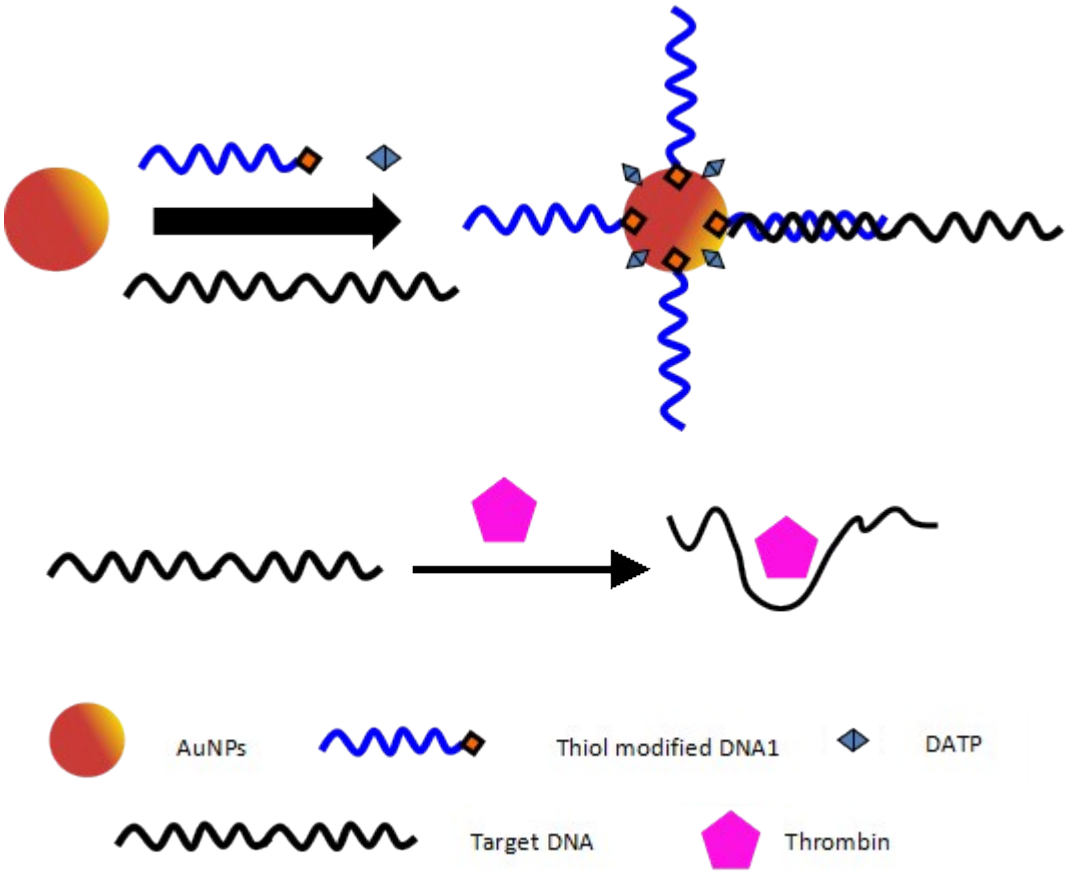


Fig. S3. Schematic illustration of the forming of DNA1-AuNPs-apatmer compounds and the recognization between apatmer and thrombin.

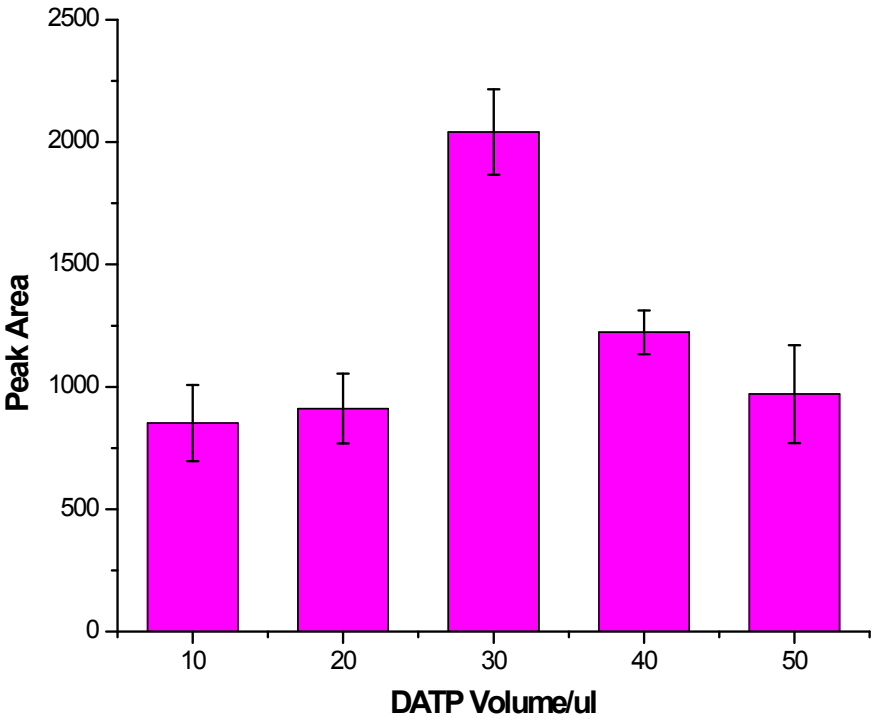


Fig. S4. Effect of volumes of DATP on the peak areas of red bands on the test zone for 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.

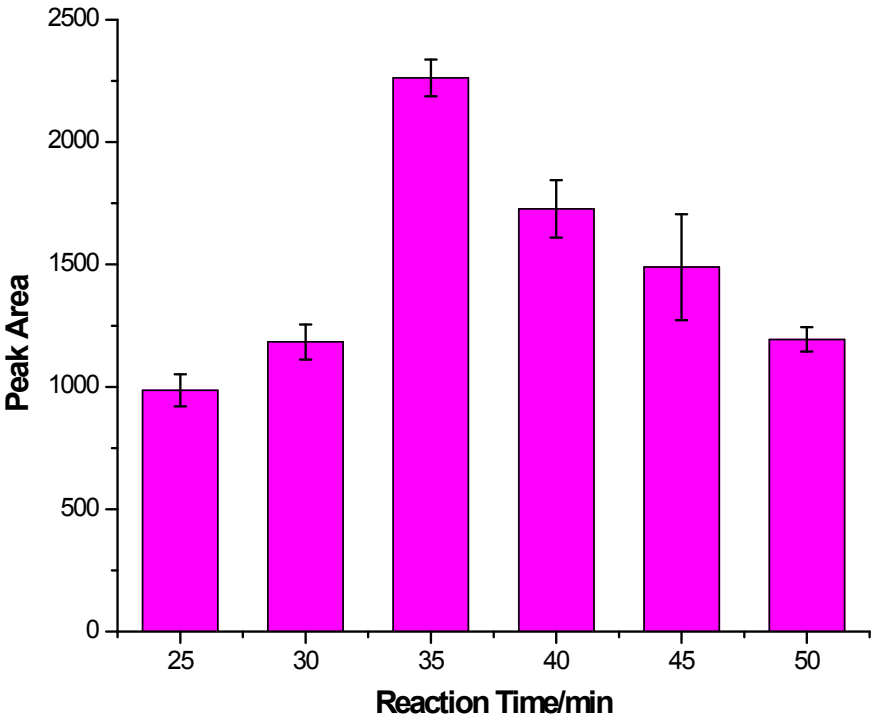


Fig. S5. Effect of different reaction time of DNA1-AuNPs-apatmer and DATP on the responses of 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.

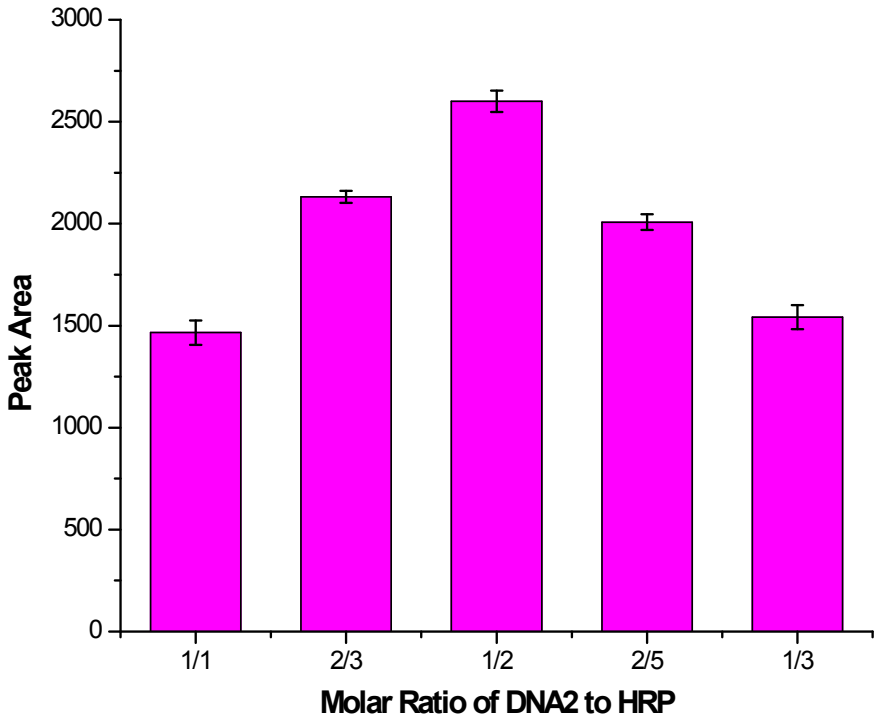


Fig. S6. Effect of different molar ratios of DNA2 to HRP (1/1, 2/3, 1/2, 2/5, and 1/3) in the DNA2-AuNPs-HRP compounds. The error bars represent the standard deviation of ten independent measurements.

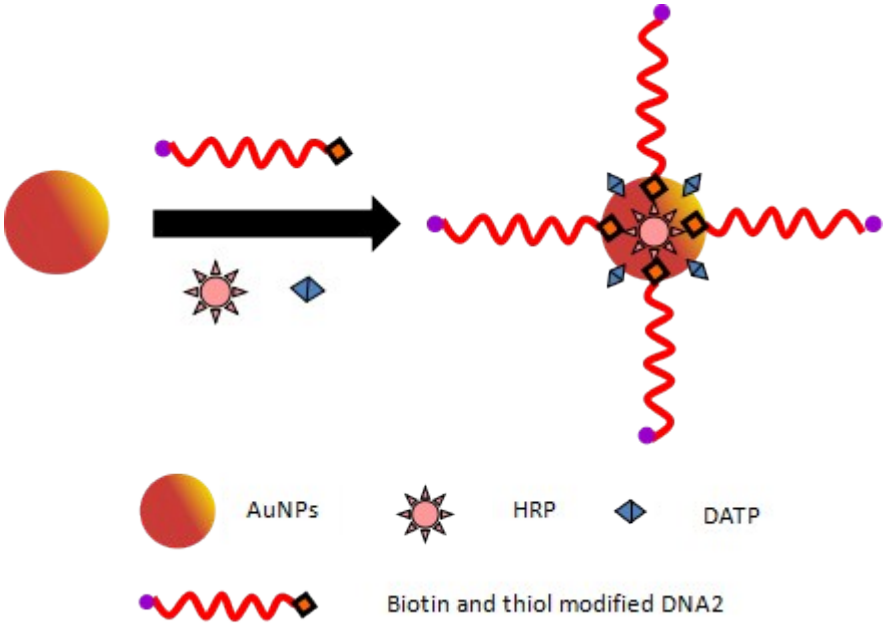


Fig. S7. Schematic illustration of the forming of DNA2-AuNPs-HRP compounds.

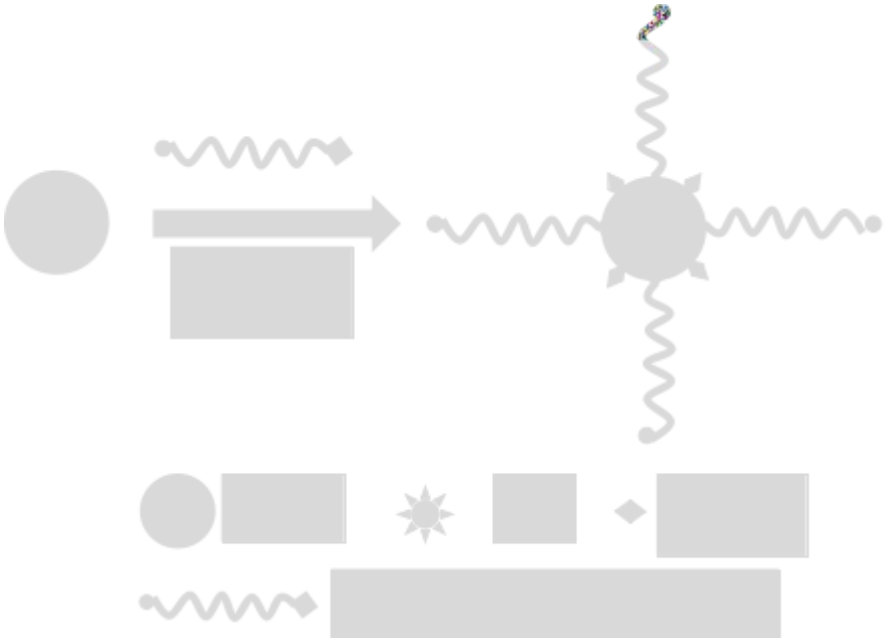


Fig. S8. Schematic illustration of the forming of DNA2-AuNPs compounds.

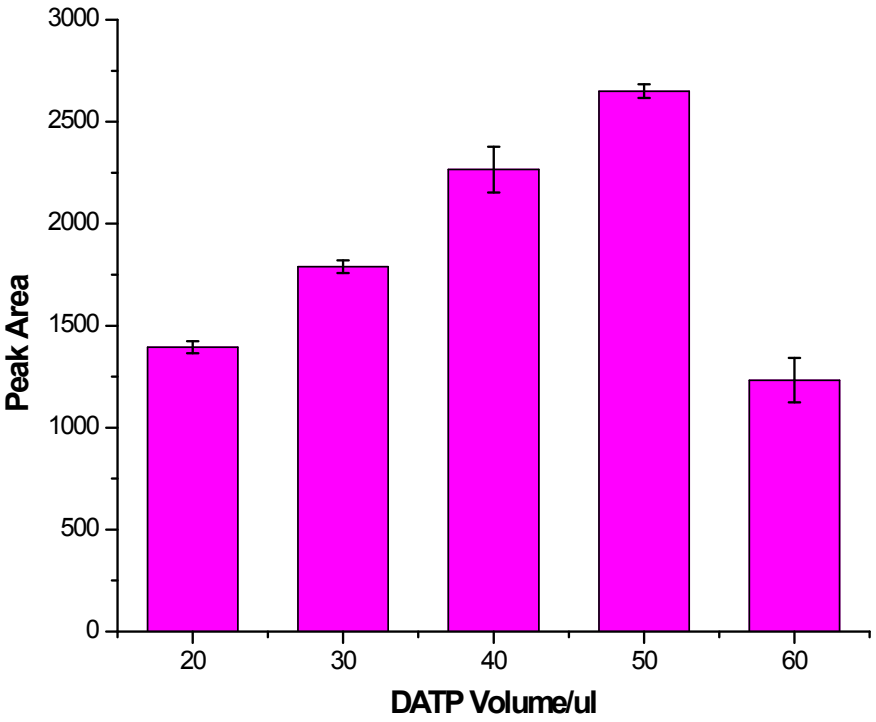
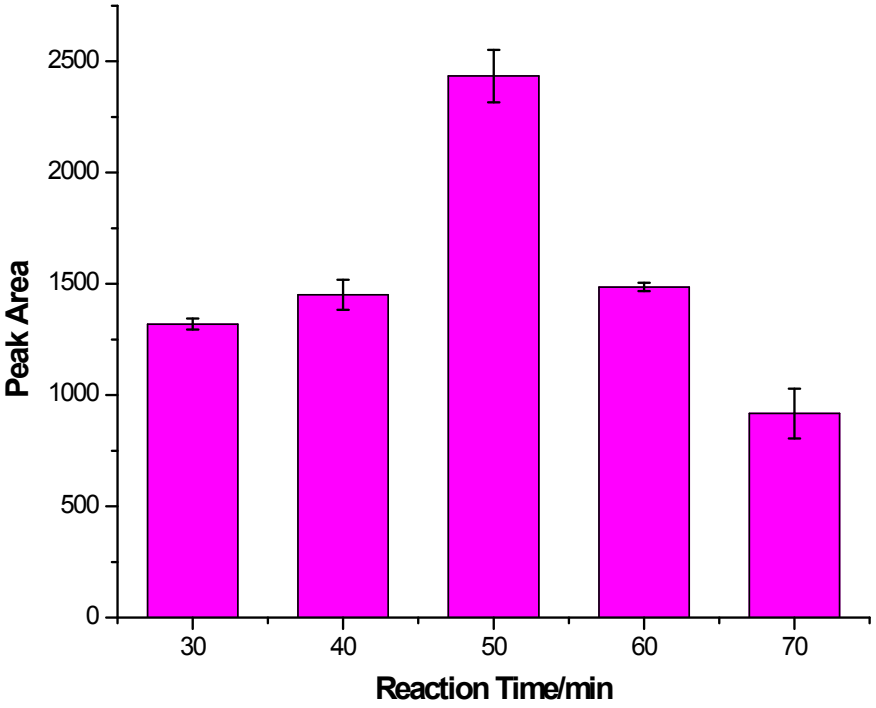


Fig. S9. Effect of volumes of DATP on the peak areas of red bands on the test zone for 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.



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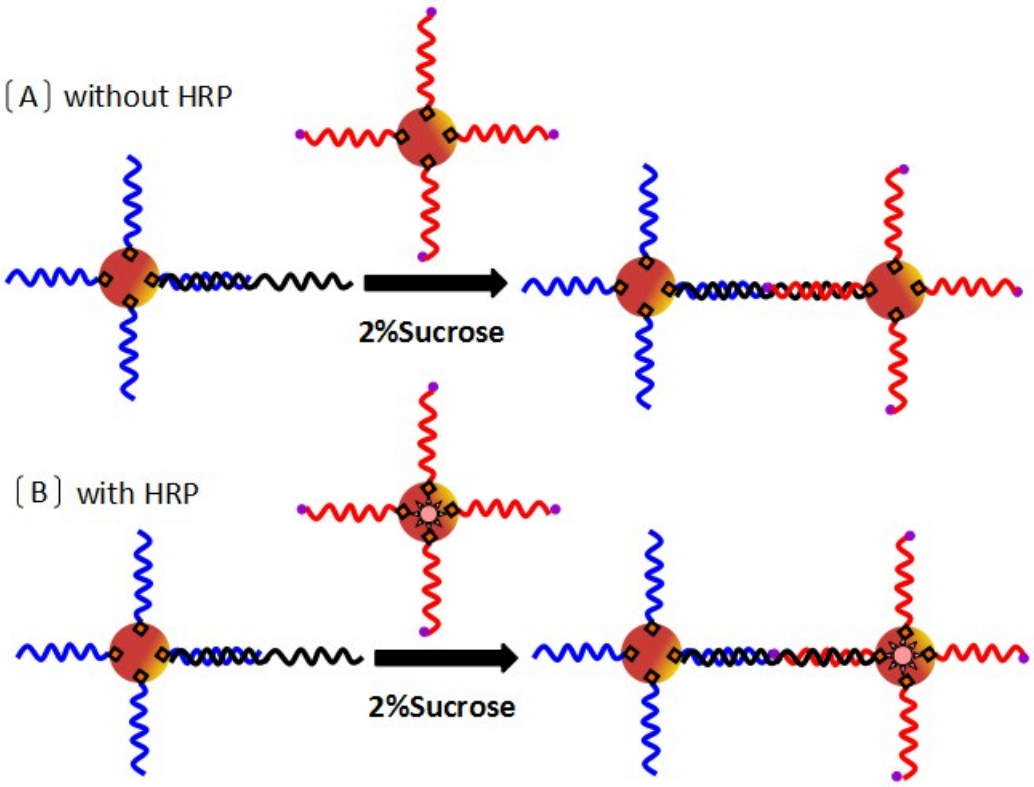


Fig. S11. Schematic illustration of the forming of aggregates (A) without HRP (B) with HRP.

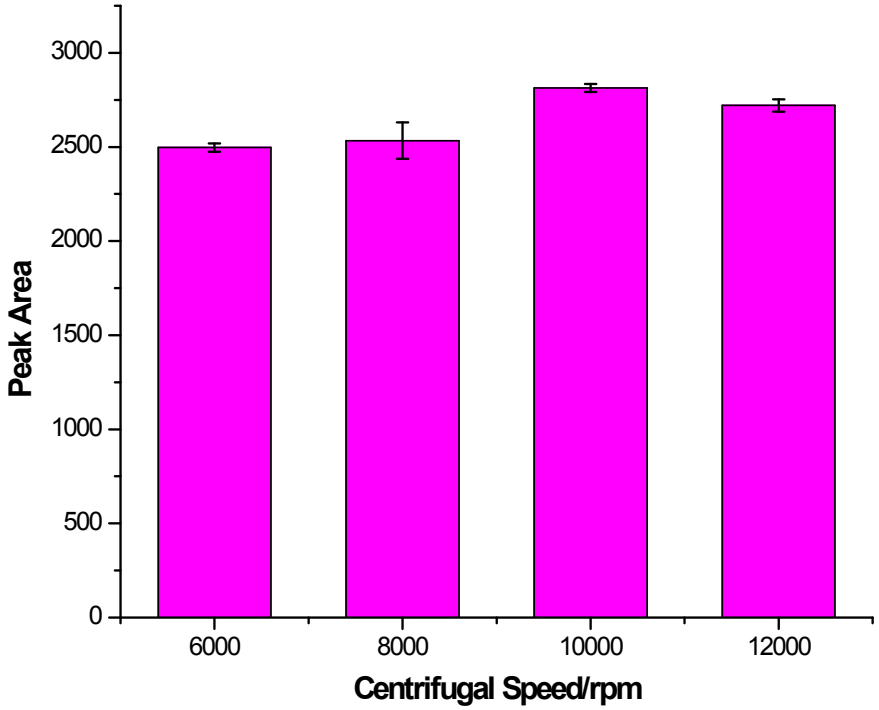


Fig. S12. Effect of different centrifugal speed for the forming of aggregates. The error bars represent the standard deviation of ten independent measurements.

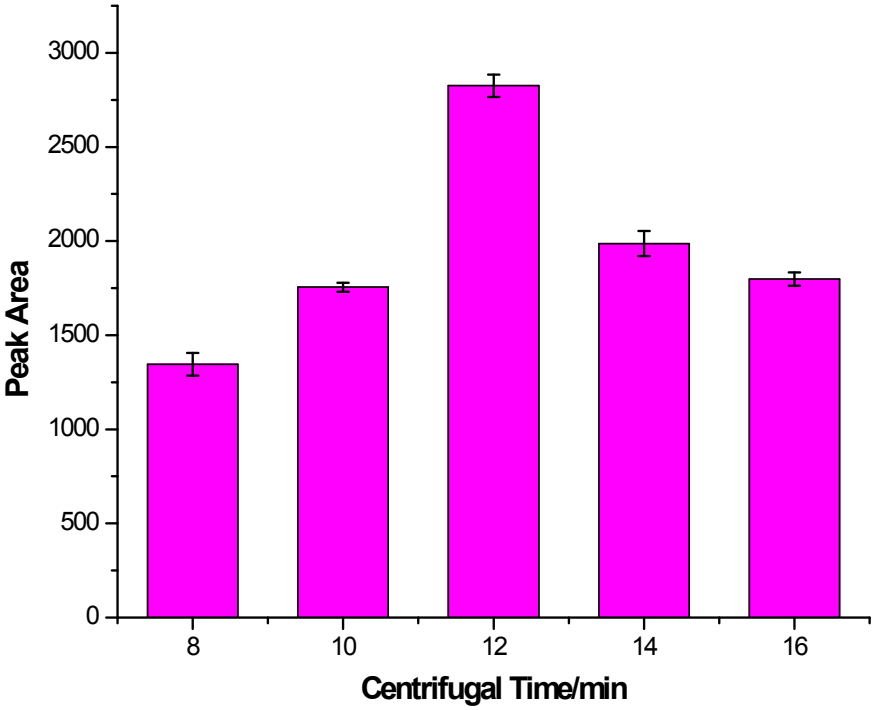


Fig. S13. Effect of centrifugal time for the forming of aggregates. For: 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.

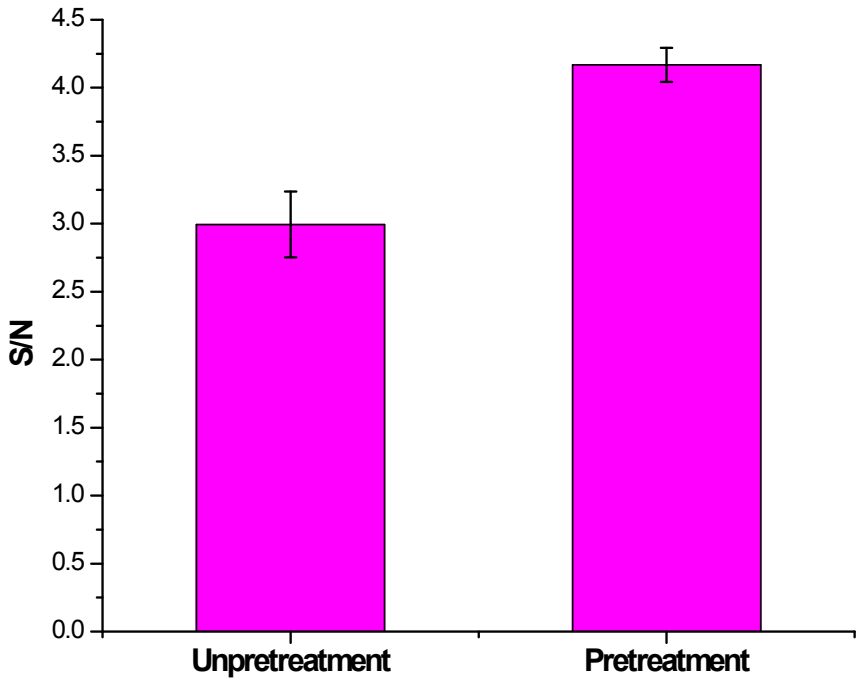


Fig. S14. Plots of the signal to noise of peak areas of red bands on the test zone vs different treatment methods of the sample pad. For: 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.

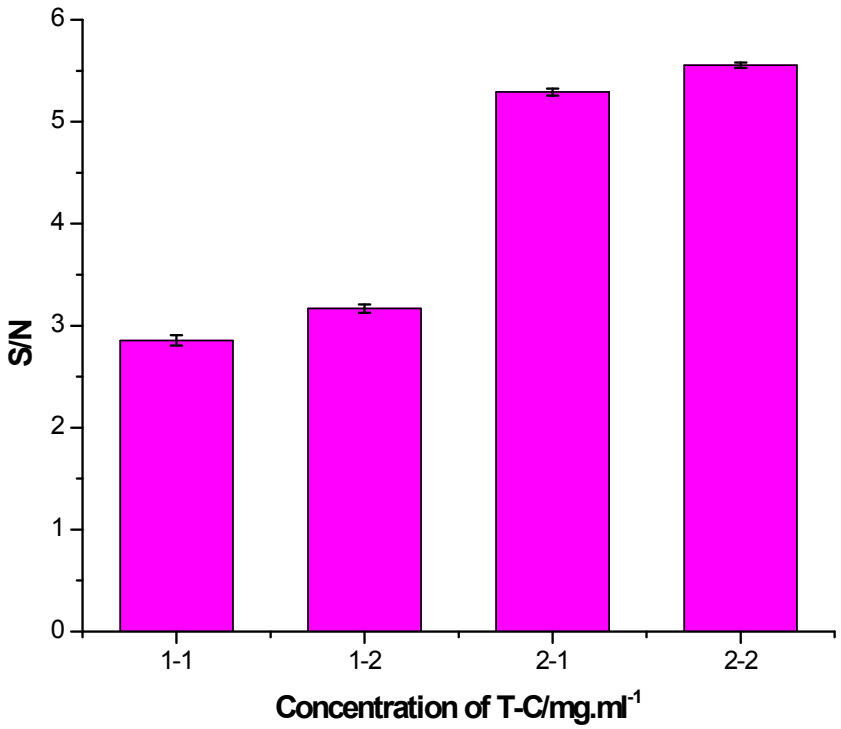


Fig. S15. Plots of the signal to noise of peak areas of red bands on the test zone vs different treatment methods of the nitrocellulose. For: 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.

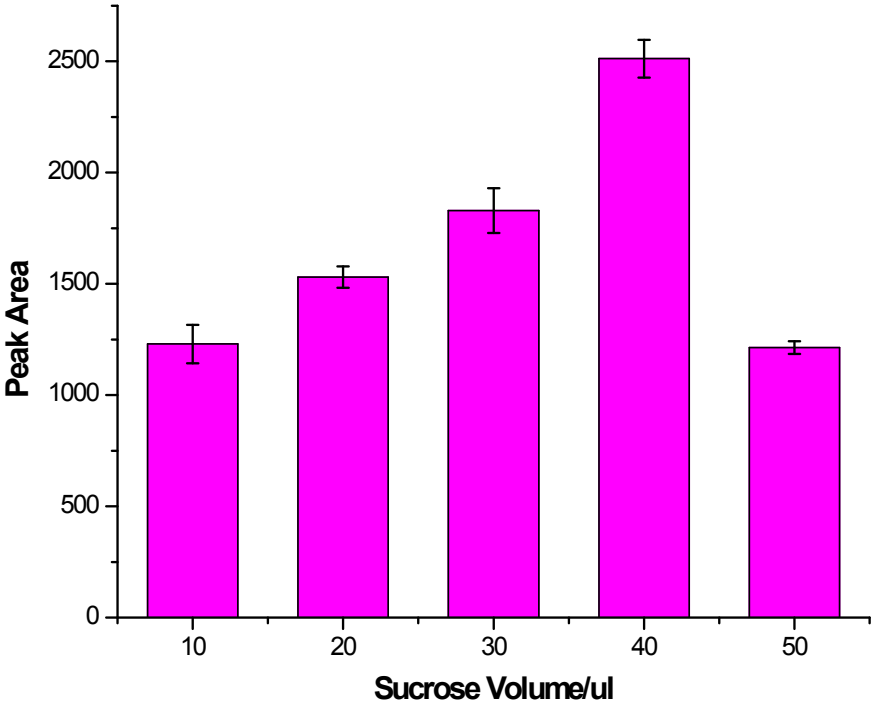
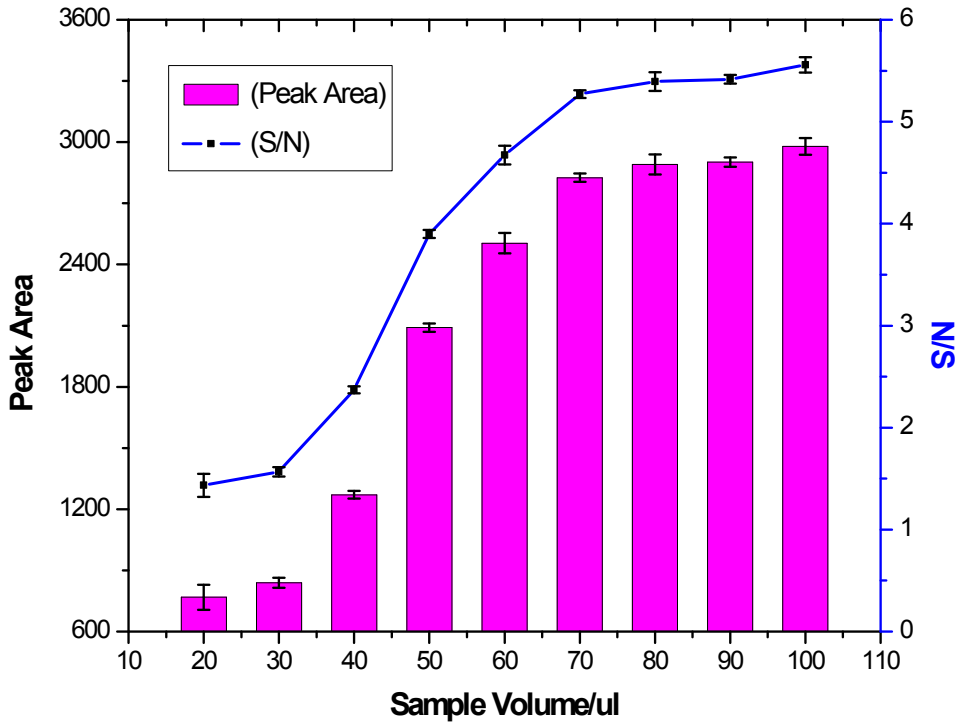


Fig. S16. Plots of the peak areas of red bands on the test zone vs different volumes of 2% sucrose for 20 nM thrombin. The error bars represent the standard deviation of ten independent measurements.



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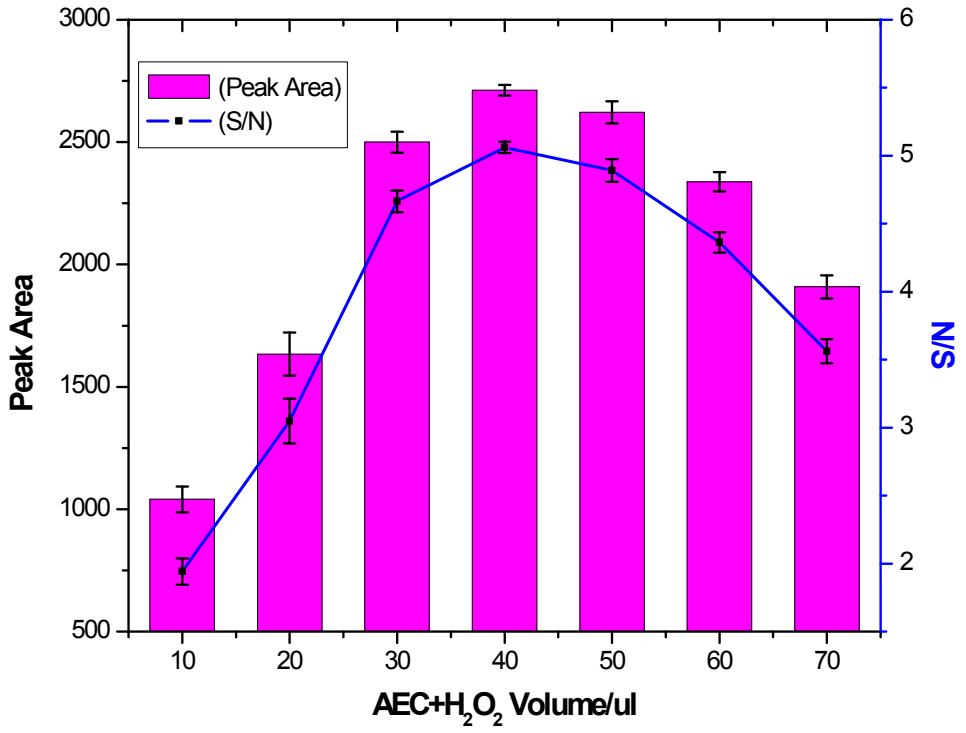

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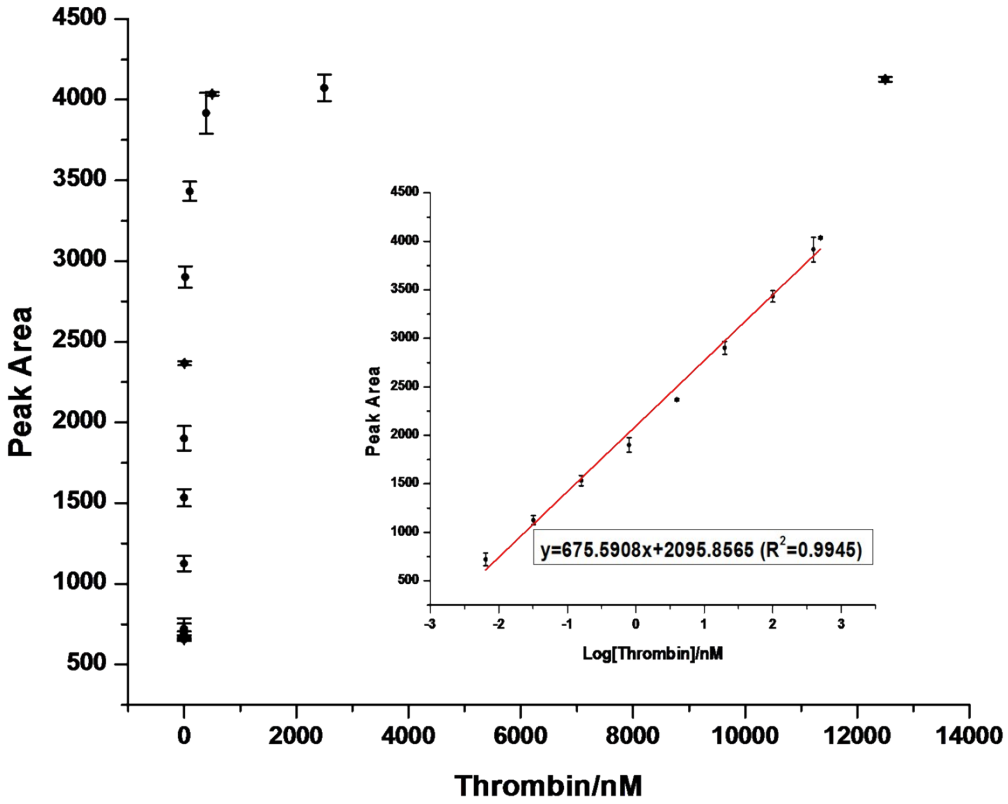

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References

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