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Stability and Bioactivity of Thrombin Binding Aptamers Modified with D-/L-Isothymidine in the Loop Regions

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The synthesize of the TBA varients

TBA and **D-/L-isoT** modified **TBAs** were synthesized on ABI 394 automated RNA/DNA synthesizer (**IsoT** phosphoramidite monomers and **isoT**-modified oligonucleotides were synthesized by our lab according to the literature[1, 2] using standard phosphoramidite chemistry). All the **TBA** variants were purified by C18 reverse high performance liquid chromatography (XBridgeTM OST C18, 2.5 μm, 10 mm × 50 mm) using a linear gradient of 5→30% eluent A in 30 min. Solutions of 0.1 M Et_3N-CH_3COOH in water, pH = 7.7, were used as eluent B, and CH_3CN was used as eluent A. Then the isolated DMT-on oligonucleotides were treated with 80% acetic acid for 10 min at room temperature. After neutralization with Et_3N, the oligonucleotide solutions were desalted by Sephadex G25

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column respectively. The purity of all oligonucleotides was verified by Capillary Gel Electrophoresis and polypropylene gel electrophoresis to be over 90%, and the oligonucleotide compositions were confirmed by MALDI-TOF-MS spectrometry.

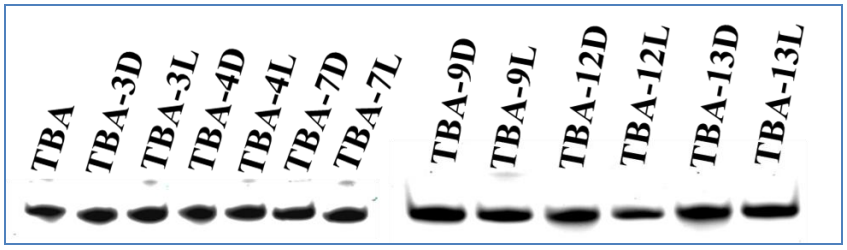


Figure S1. The purity of synthesized oligonucleotides determined by polyacrylamide gel electrophoresis.

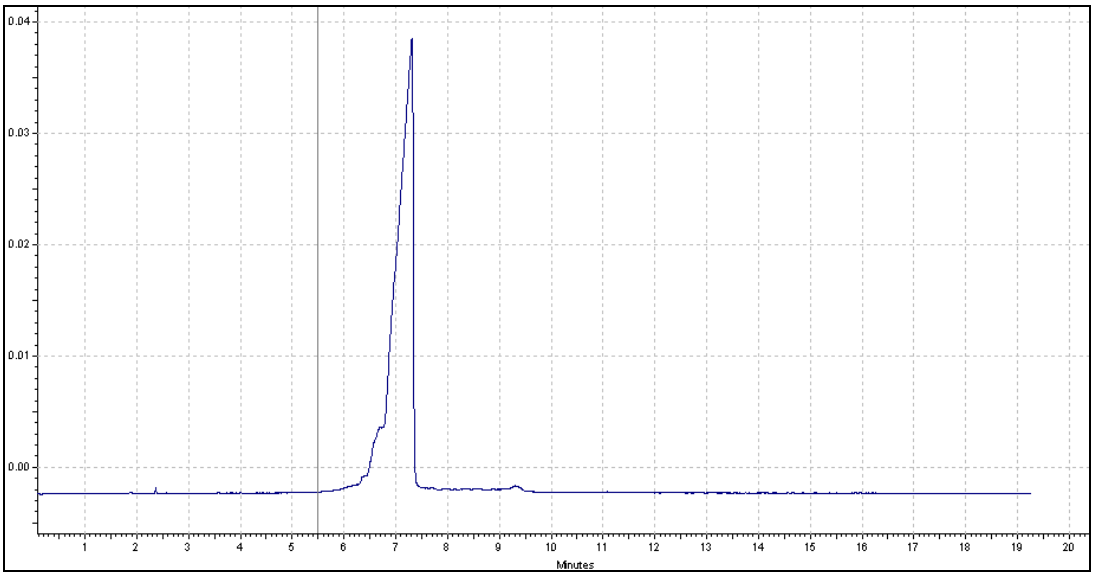


Figure S2. The purity of synthesized oligonucleotides determined by capillary gel electrophoresis. Instrument and reagents: Beckman PA800 capillary electrophoresis (Beckman, USA), 32 Karat software (Version 5.0, Beckman) workstation; capillary (inner diameter 100 nm, OD 375 nm, the effective length 20 cm); Capillary Coating: 4% polypropylene amide. Separation conditions: 25 mmol / L Tris-boric-EDTA buffer solution (pH 7.94), 20% (w/v) PEG 35000, 25 ℃, 25 kV, Detection wavelength was 254 nm.

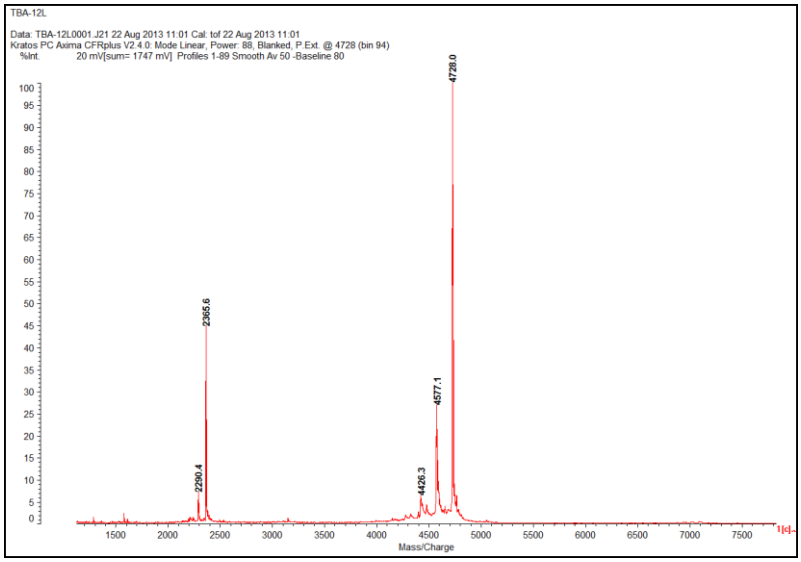


Figure S3. MALDI-TOF-MS of **TBA** and its variants. Found: 4728 $[M+2H]^+$; Calcd: 4726.

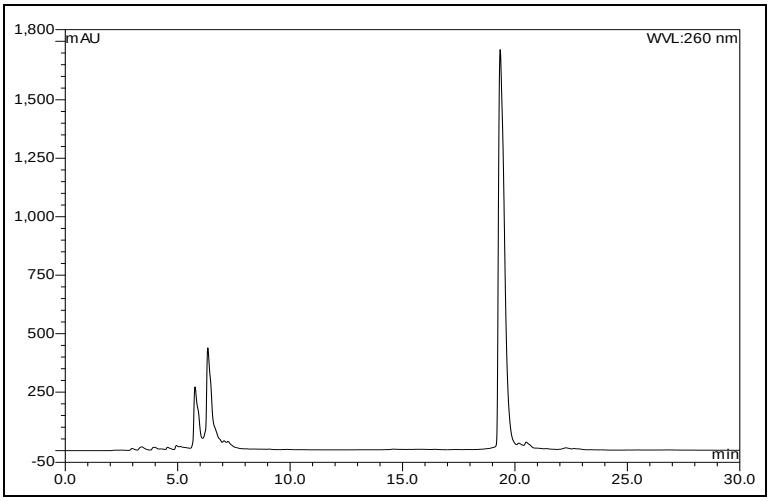


Figure S4. The separation results of **TBA** variants with HPLC. Dionex UltiMate 3000 HPLC, XBridgeTM OST C18 column (2.5 μm, 10 mm × 50 mm), Gradient program: 5−30% eluent A in 30 min(A: 0.1M Et_3N-CH_3COOH in water, pH = 7.7; B: CH_3CN), column temperature 40 °C.

Table S1. **TBA** and D/L-**isoT** modified variants

Name	Sequence(5'-3')	MALDI-TOF-MS $[M+2H]^+$	
		Calcd.	Found
TBA	GGT TGG TGT GGT TGG	4726	4728
TBA-3D	GGT_D TGG TGT GGT TGG	4726	4728
TBA-3L	GGT_L TGG TGT GGT TGG	4726	4728
TBA-4D	GGT T_DGG TGT GGT TGG	4726	4728
TBA-4L	GGT T_LGG TGT GGT TGG	4726	4728
TBA-7D	GGT TGG T_DGT GGT TGG	4726	4728
TBA-7L	GGT TGG T_LGT GGT TGG	4726	4728

TBA-9D	GGT TGG TG***T*** $_D$ GGT TGG	4726	4728
TBA-9L	GGT TGG TG***T*** $_L$ GGT TGG	4726	4728
TBA-12D	GGT TGG TGT GGT_D TGG	4726	4728
TBA-12L	GGT TGG TGT GG***T*** $_L$ TGG	4726	4728
TBA-13D	GGT TGG TGT GGT ***T*** $_D$GG	4726	4728
TBA-13L	GGT TGG TGT GGT ***T*** $_L$GG	4726	4728
TBA-3L7D	GG ***T*** $_L$ TGG ***T*** $_D$GT GGT TGG	4726	4728
TBA-3L9L	GG ***T*** $_L$ TGG TG ***T*** $_L$ GGT TGG	4726	4728
TBA-3L 12L	GGT TGG TGT GG***T*** $_L$ ***T*** $_L$ GG	4726	4728
TBA-7D9L	GGT TGG ***T*** $_D$G T$_L$ GGT TGG	4726	4728
TBA-7D 12L	GGT TGG ***T*** $_D$GT GG ***T*** $_L$ TGG	4726	4728
TBA-9L 12L	GGT TGG TG ***T*** $_L$ GG ***T*** $_L$ TGG	4726	4728

Note: The red italics represent D-**isoT** modification and the blue italics represent **L-isoT** modification

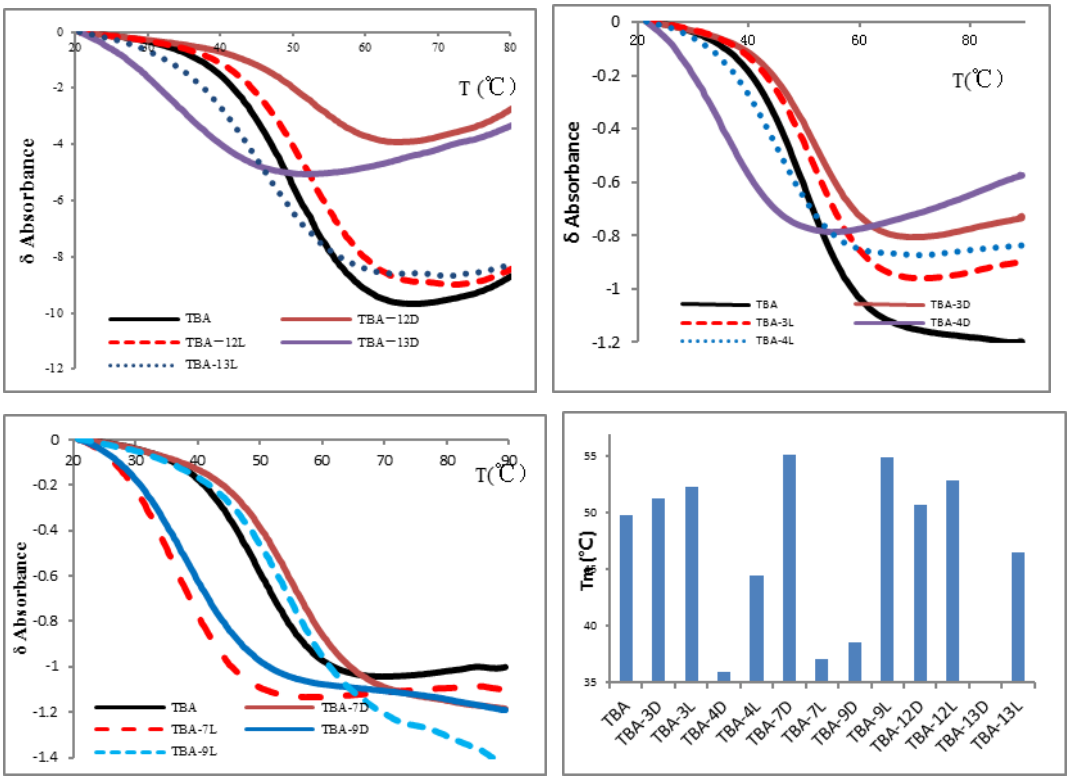
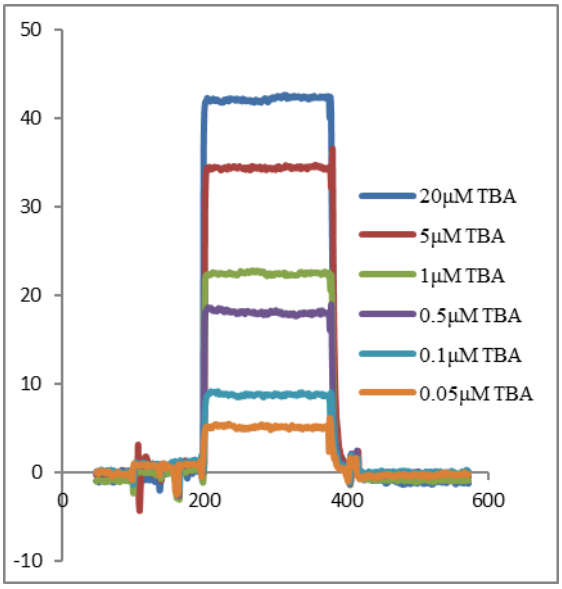
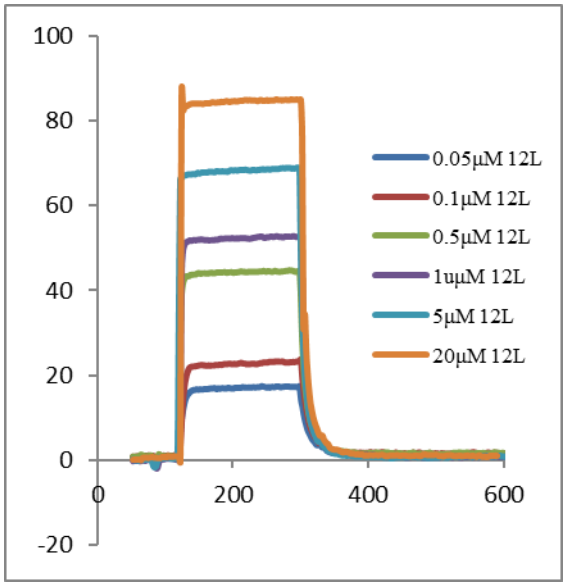
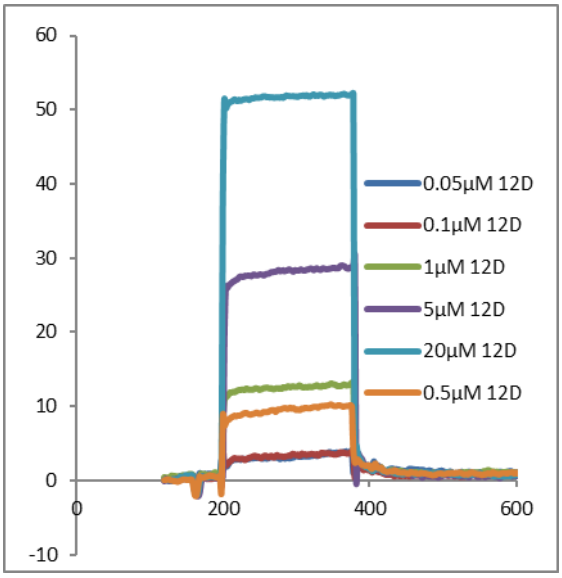
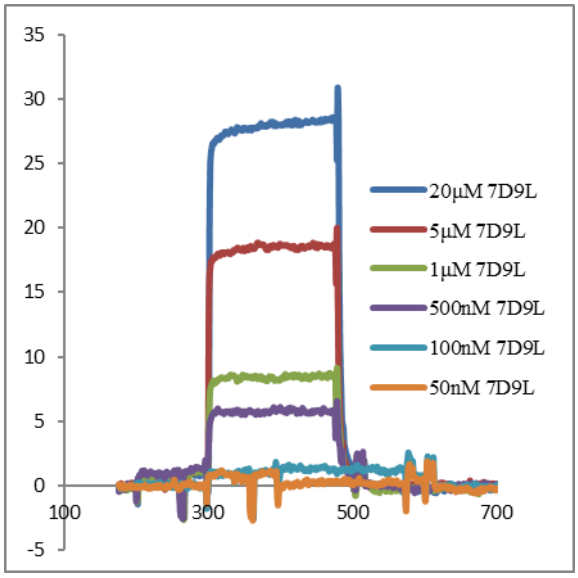


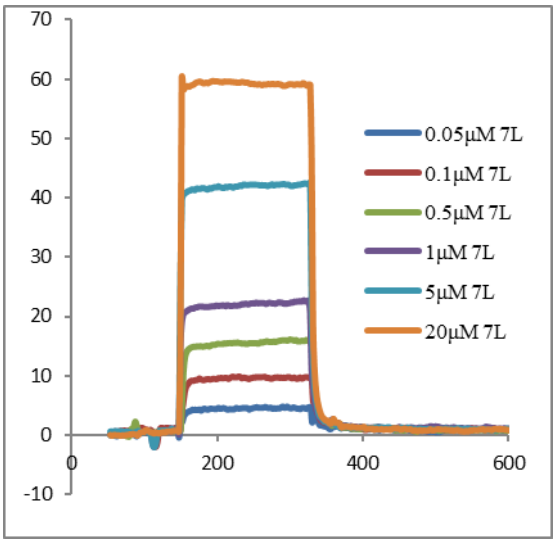
Figure S5. The thermodynamic stability of **D-/L-isoT** modified **TBA**s. The oligonucleotides were dissolved in the Tm buffer (100 mM potassium chloride and 10 mM sodium cacodylate, pH 7.4) and the concentration was 8 μM.


20μM TBA
5μM TBA
1μM TBA
0.5μM TBA
0.1μM TBA
0.05μM TBA


0.05μM 12L
0.1μM 12L
0.5μM 12L
1uμM 12L
5μM 12L
20μM 12L


0.05μM 12D
0.1μM 12D
1μM 12D
5μM 12D
20μM 12D
0.5μM 12D


20μM 7D9L
5μM 7D9L
1μM 7D9L
500nM 7D9L
100nM 7D9L
50nM 7D9L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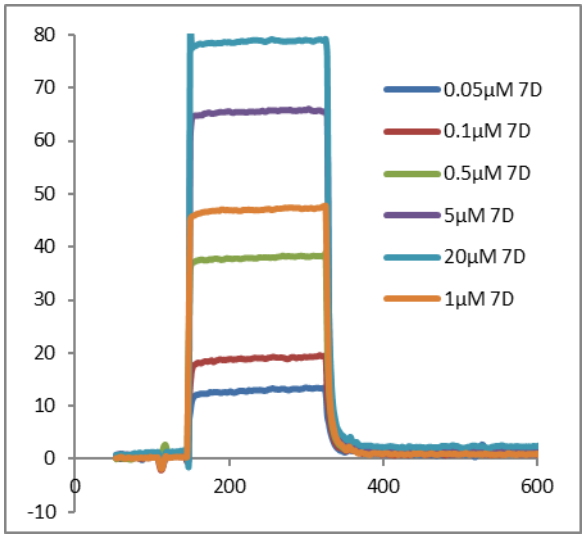
0.1μM 7L
0.5μM 7L
1μM 7L
5μM 7L
20μM 7L


0.05μM 7D
0.1μM 7D
0.5μM 7D
5μM 7D
20μM 7D
1μM 7D

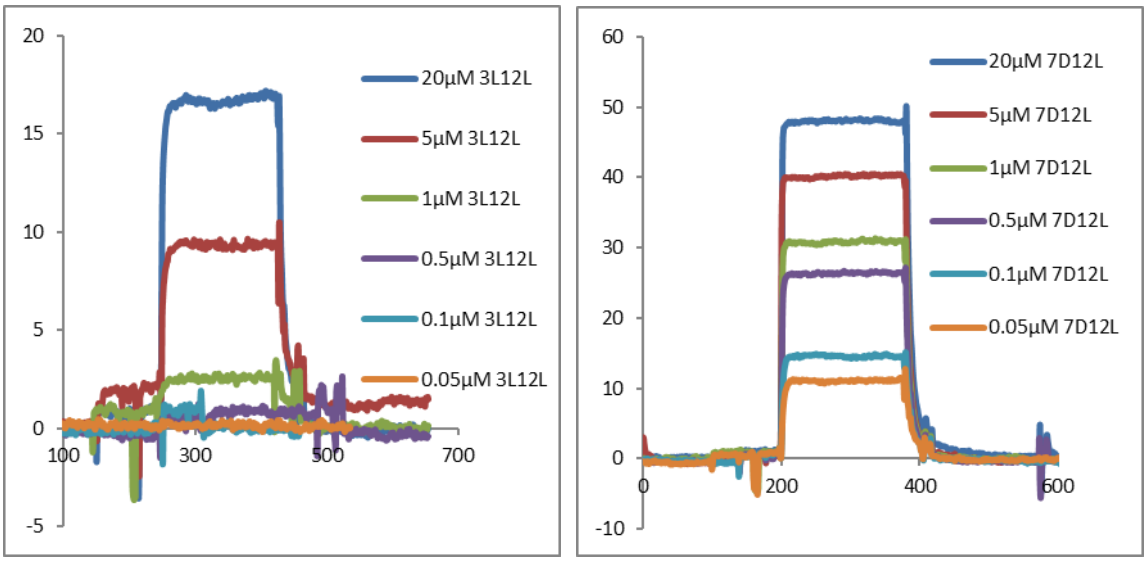
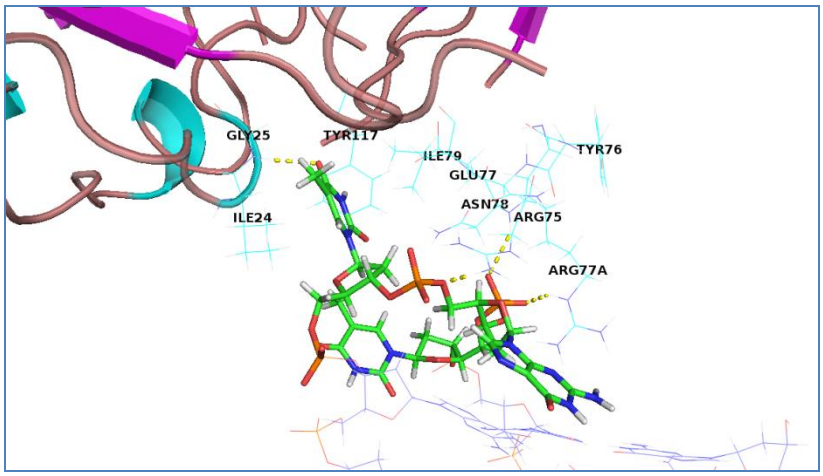
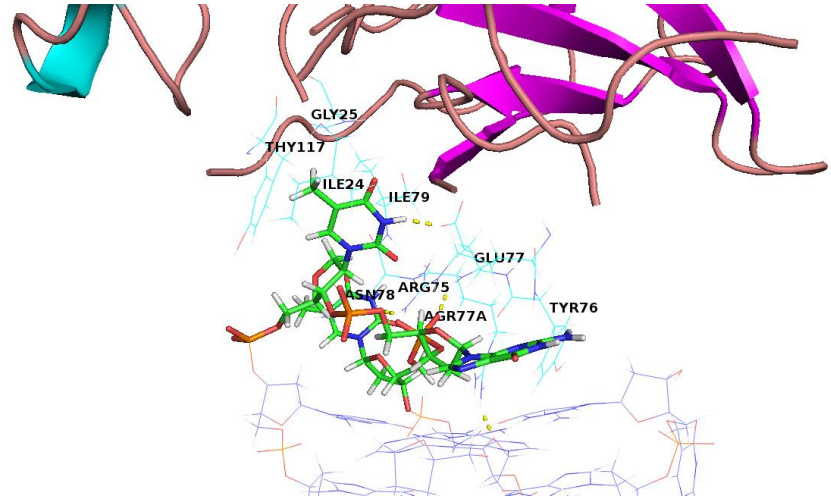


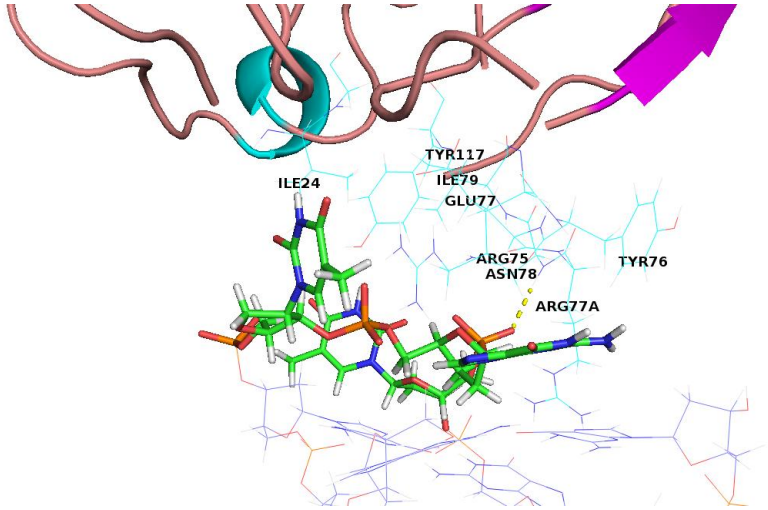
Figure S6. SPR results of natural and **D-/L-isoT** modified **TBAs** with thrombin



A. TBA/thrombin



B. TBA-7D/thrombin



C. TBA-7L/thrombin

Figure S7. The respective binding modes of **TBA**, **TBA-7D**, **TBA-7L** with thrombin

References

1. Z. J. Yang, H. W. Yu, J. M. Min, L. T. Ma, L. H. Zhang, *Tetrahedron: Asymmetry* **1997**, *8*, 2739.
2. J. Chen, L. R. Zhang, J. M. Min, L. H. Zhang, *Nucleic Acids Res.*, **2002**, 3005.