Supplementary Material for

Several structural motifs cooperate in determining the highly effective anti-thrombin activity of NU172 aptamer

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Supplementary Tables

Table S1. Diffraction data statistics.

	Thrombin–NU172–K	Thrombin–NU172–Na
Space group	I222	I222
Unit-cell parameters		
a (Å)	67.88	67.33
b (Å)	113.60	120.69
c (Å)	208.03	208.94
α (deg)	90.00	90.00
β (deg)	90.00	90.00
γ (deg)	90.00	90.00
Resolution limits (Å)	104.02-2.50 (2.59-2.50)[a]	104.51-2.80 (2.95-2.80)[a]
No. of observations	1816277	84545
No. of unique reflections	26684	21199
Completeness (%)	93.8 (64.7)	99.0 (99.4)
$\langle I/\sigma(I)\rangle$	14.7 (2.6)	12.4 (3.9)
Average multiplicity	5.3 (3.1)	4.0 (4.2)
R_{merge}[b] (%)	7.9 (31.7)	6.7 (34.0)
V_M (Å^3 Da^{-1})	4.47	4.73
No. of molecules in the asymmetric unit	1	1
Solvent content (%)	74.7	76.1

[a] Values in brackets refer to the highest resolution shell.

[b] $R_{merge} = \sum_{hkl}\sum_{I}|I_i(hkl)-\langle I(hkl)\rangle|/\sum_{hkl}\sum_{i}I_i(hkl)$, where $I_i(hkl)$ is the *i*th intensity measurement of the reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

Table S2. Summary of refinement statistics.

	Thrombin–NU172–K	Thrombin–NU172–Na
Resolution (Å)	104.02-2.50	104.51-2.80
No. of reflections	25367	20089
R_{factor}/R_{free}[a]	0.169/0.205	0.159/0.203
No. of atoms		
Total	3183	3146
Protein	2367	2360
Aptamer	543	543
Ions	2	2
Carbohydrates	106	95
Waters	129	110
Average *B* factor (Å^2)		
Overall	49.15	53.14
Protein	42.22	45.61
Aptamer	78.47	77.40
Ions	47.98	41.95
Carbohydrates	70.56	116.73
Waters	38.74	42.66
RMSD from ideal values		
Bond lengths (Å)	0.01	0.01
Bond angles (deg)	1.48	1.18
Ramachandran plot, residues in (%)		
Most favoured region	97.2	95.8
Additionally allowed region	2.8	4.2
Generously allowed region	0	0
PDB code	6EVV	6GN7

[a] $R_{factor} = \sum_{hkl}||F_o|-|F_c||/\sum_{hkl}|F_o|$, where F_o and F_c represent the observed and calculated structure factors, respectively. The R_{factor} was calculated using 95% of the data, which were included in refinement, and R_{free} was calculated using 5% of the data, which were excluded from refinement.

Table S3. Comparison of interface interactions and areas among thrombin-TBA-K, -TBA-Na, -mTBA, -RE31 (PDB codes: 4DII, 4DIH, 3QLP, 5CMX, respectively), -NU172-K and -NU172-Na complexes, calculated by Contact, CoCoMaps and PISA programs. The threshold distance to select interacting residues was 3.9 Å and 5.0 Å in Contact and in CoCoMaps, respectively.

	TBA-K	TBA-Na	mTBA	RE31	NU172-K	NU172-Na
Contact						
Thy A/Gua (in A-region)	Ile24 His71 Ile79 Tyr117	Ile24 Arg75 Glu77 Ile79 Tyr117	Ile24 Arg75 Glu77 Ile79 Tyr117	Ile24 Glu77 Ile79 Tyr117	Ile24 Gly69 His71 Arg75 Glu77 Ile79 Tyr117	Ile24 His71 Arg75 Glu77 Ile79 Tyr117
Thy B (in A-region)	Arg75 Arg77A Asn78 Ile79	Arg75 Arg77A Asn78 Ile79	Arg75 Arg77A Asn78 Ile79	Arg75 Arg77A Asn78 Ile79	Arg75 Arg77A Asn78 Ile79	Arg75 Arg77A Asn78 Ile79
Thy C (in B-region)	Tyr76	Tyr76 Ile82	Gln38 Leu65 Tyr76	Tyr76	Tyr76 Ile82	Tyr76 Ile82
Thy D (in B-region)	Thr74 Arg75 Tyr76 Arg77A	Arg75 Tyr76 Arg77A	Arg75 Tyr76 Arg77A	Arg75 Tyr76 Arg77A	Arg75 Tyr76 Arg77A	Arg75 Tyr76 Arg77A
Gua (G-quadruplex)	Arg75 Arg77A Asn78	Thr74 Arg75 Arg77A	Arg75 Arg77A Asn78	Thr74 Arg75 Arg77A Asn78	Arg75 Arg77A Asn78	Arg75 Arg77A
Gua (GTA loop)					Arg75	Arg75
CoCoMaps						
Interface area (Å²)	540	563	663	553	591	588
Polar Interface area (Å²)	269	288	337	283	316	316
Non polar Interface area (Å²)	271	276	326	270	274	272
N° interacting residues thrombin	10	11	14	12	13	13
N° interacting residues aptamer	8	8	8	8	9	9
N° hydrophilic-hydrophobic interaction	3	5	6	5	5	5
N° hydrophilic-hydrophilic interaction	18	20	23	20	22	21
PISA						
Interface area (Å²)	543	565	657	552	590	589
H-bonds	11	12	11	10	11	9

Table S4. Anticoagulant properties of different oligonucleotides expressed as ratio between coagulation rate in the presence of thrombin and oligonucleotide and in the only presence of thrombin.

	Thrombin:oligonucleotide ratio		
	1:1	**1:2**	**1:5**
TBA	N.D.	2	8
NU172	200	1000	N.D.
Des_NU172	N.D.	2	7
TBA_GT	N.D.	3	7

Supplementary Figures

TBA

5’-GGTTGGTGTGGTTGG-3’

NU172

5’-CGCCTAGGTTGGGTAGGGTGGTGGCG-3’

Des_NU172

5’-GGTTGGGTAGGGTGG-3’

TBA_GT

5’-GGTTGGTGTGGGTGG-3’

Figure S1. Sequences of the different oligonucleotides.

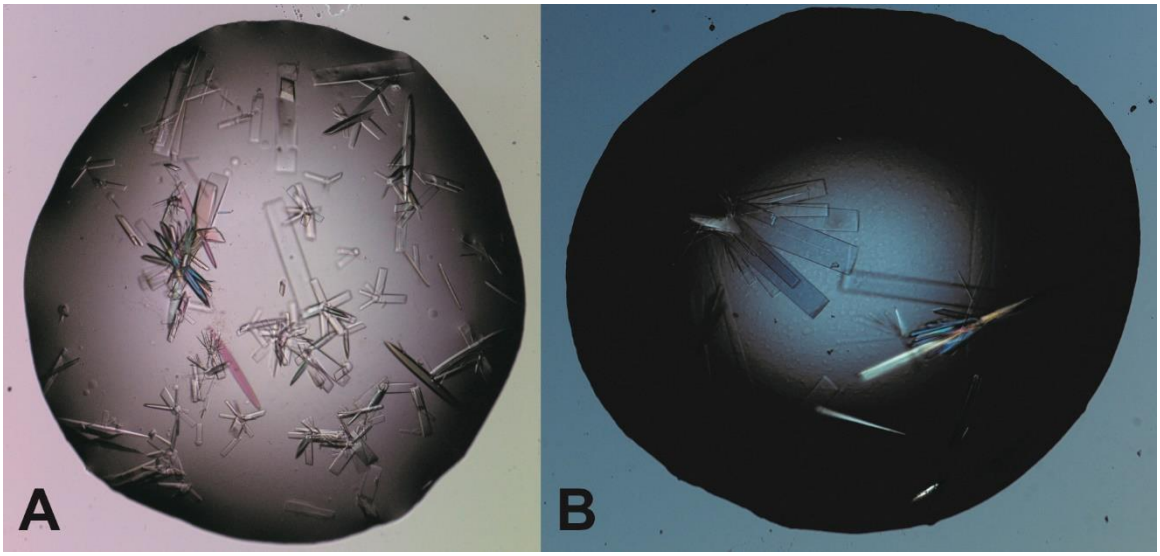

Figure S2. Crystals of thrombin–NU172–K (A) and thrombin–NU172–Na (B) complexes, grown in 50% (*v/v*) Tacsimate™ at pH 7.0. The diameter of the drops is 2.00 mm and the larger crystals are 0.50 mm x 0.07 mm, approximately.

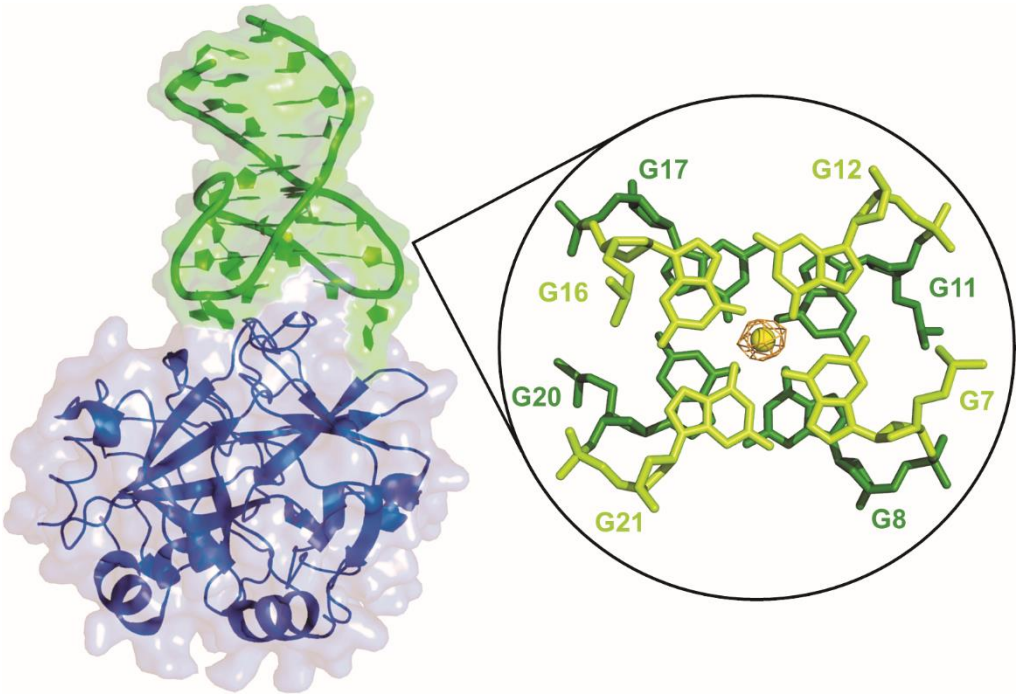

Figure S3. On the left, surface/cartoon representation of thrombin-NU172-Na complex. Thrombin is coloured in blue and the aptamer is coloured in green. On the right, view of the two tetrads along the G-quadruplex axis with the omit F_o-F_c electron density map (orange) of the sodium ion (yellow) contoured at 3.0 σ level.

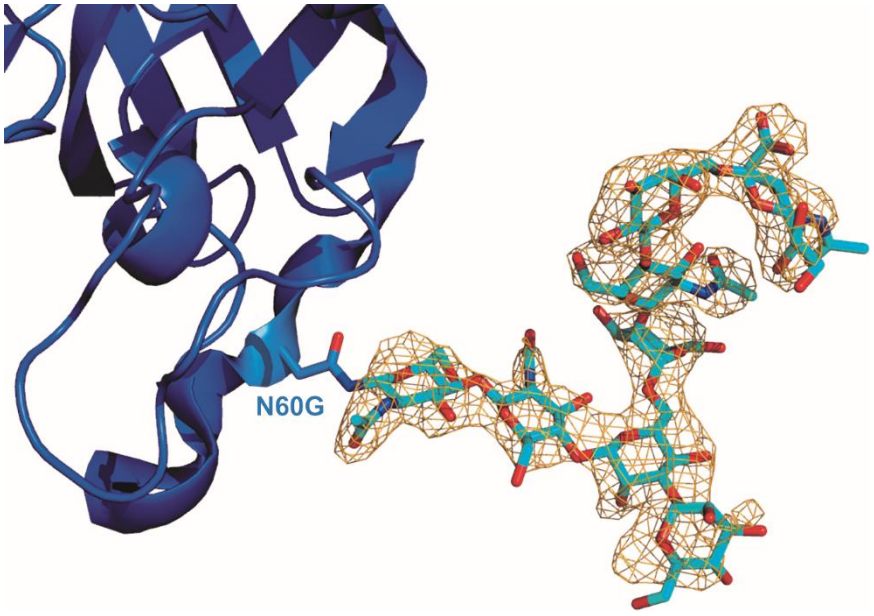

Figure S4. Omit F_o-F_c electron density map of the sugar chain linked to asparagine 60G contoured at 3.0 σ level.

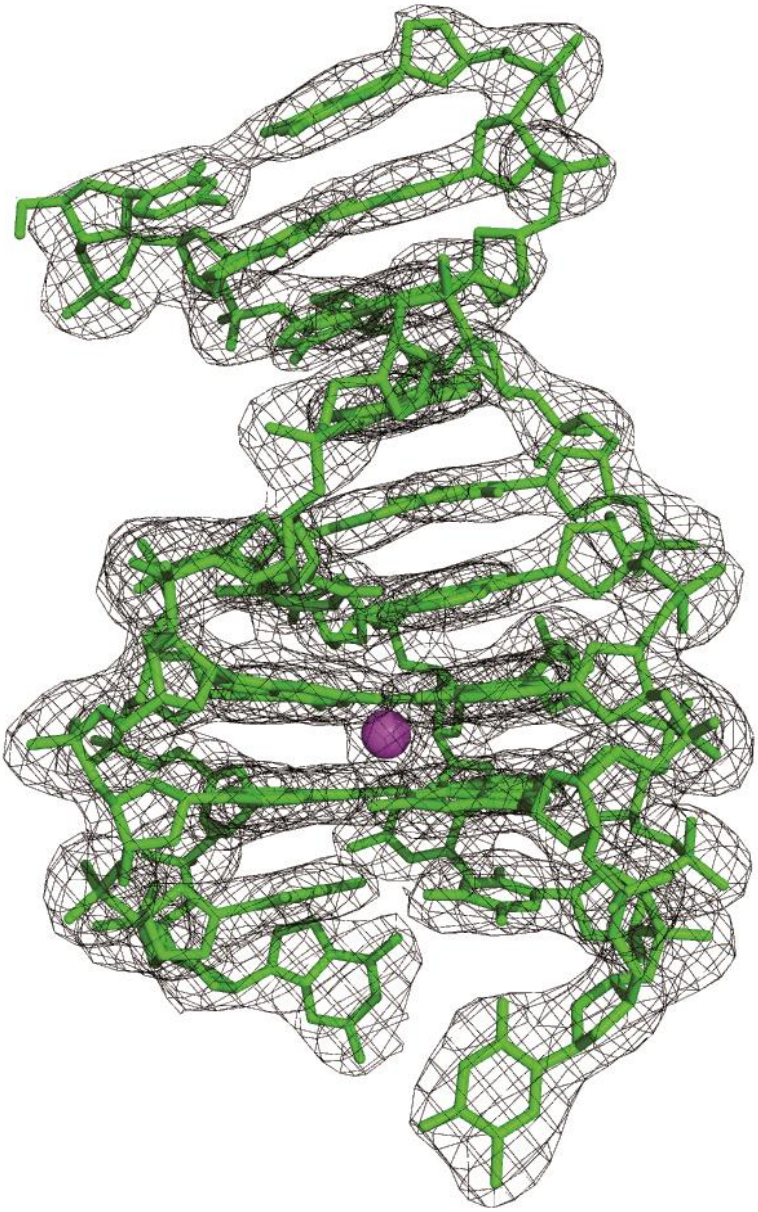

Figure S5. $2F_o$-F_c electron density map (grey) of the whole NU172 aptamer (green) in thrombin-NU172-K complex contoured at 1.0 σ level. The potassium ion between the two tetrads of the G-quadruplex is coloured in magenta.

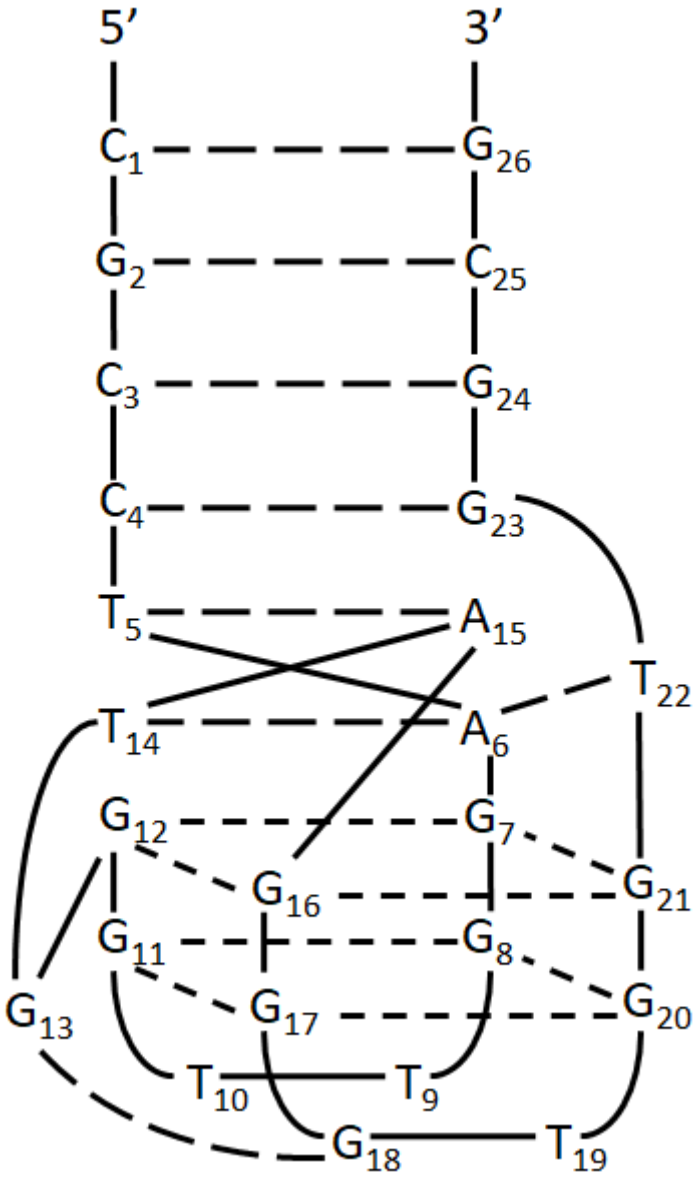

Figure S6. Schematic representation of NU172 organization when in complex with thrombin. Hydrogen bonds between bases are represented by dashed lines.

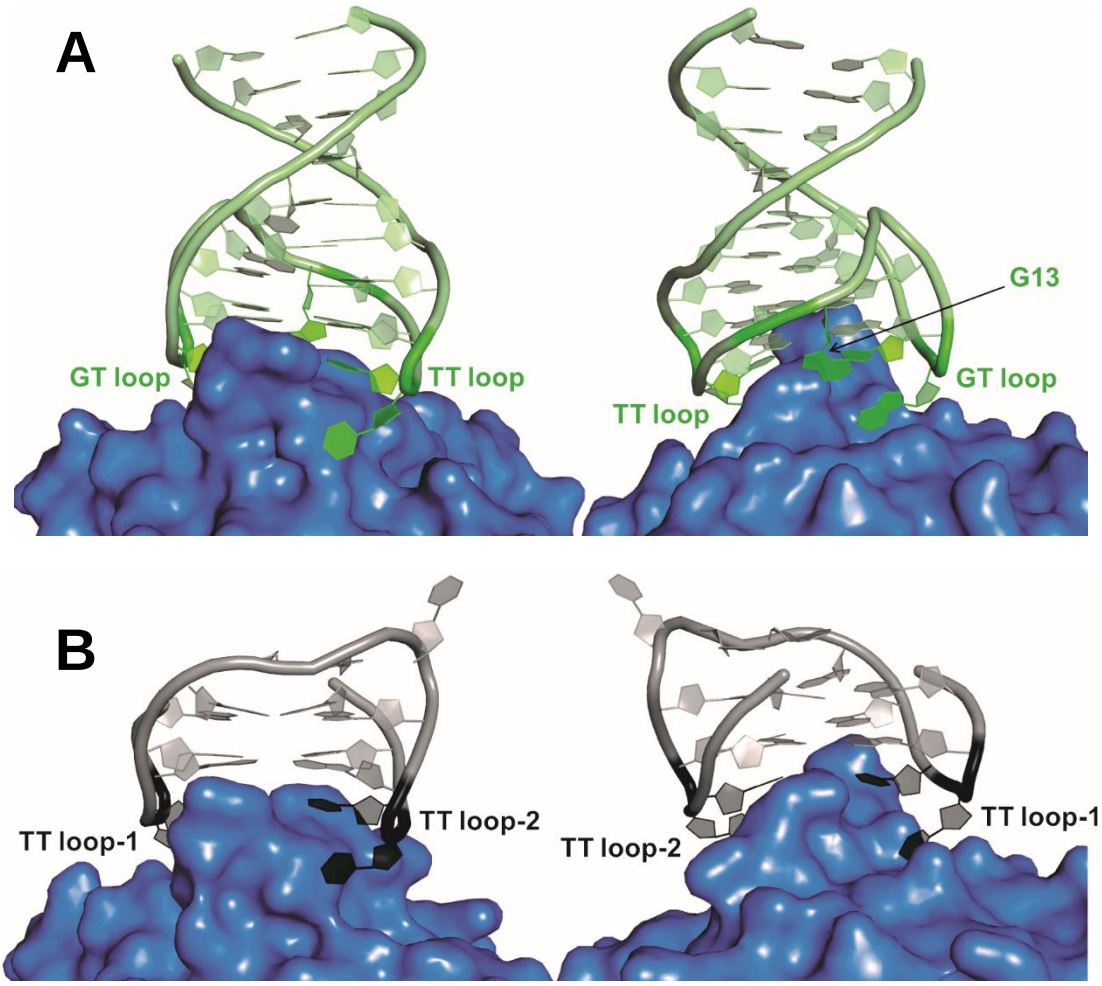

Figure S7. Cartoon/surface views of thrombin-NU172 (thrombin-NU172-K) (A) and thrombin-TBA (PDB code: 4DII) (B) interfaces. NU172 and TBA bind thrombin with a pincer-like system formed by the Thy9-Thy10 (TT loop) and the Gua18-Thy19 (GT loop) loops or by the Thy3-Thy4 (TT loop-1) and the Thy12-Thy13 (TT loop-2) loops, respectively. The interactions at B-region of exosite I, involving the TT loop of NU172 and the TT loop-2 of TBA are very similar. On the contrary, at A-region the presence in NU172 of a bulkier purine and the involvement of Gua13 (GTA loop) in the binding differentiate the two recognition motifs. Thrombin surface is coloured in blue, NU172 is coloured in green and TBA is coloured in black.