

Electronic Supplementary Information (ESI)

Light-activated thrombin catalysis and clot formation using a photoswitchable DNA aptamer

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1.1 DNA strand synthesis and characterisation

The anthracene phosphoramidite precursor was made as reported previously¹ and incorporated into the **TBA** sequence via the phosphoramidite method using an Applied Biosystems 394 DNA/RNA synthesizer.¹ All strands were purified using Reversed Phase-High-Performance Liquid Chromatography (RP-HPLC) on a Phenomenex Clarity 5 μ Oligo RP 150 x 10 mm column. A method was used where the solvent gradient ran from 0.1 M TEAA in 5% acetonitrile and 0.1 M TEAA in 15% acetonitrile before a 100% acetonitrile flush. Once the samples were collected they were dried *in vacuo* before being dissolved in 1.0 mL of MilliQ water. The samples were desalted using a NAP-10 column (GE Healthcare) and eluted in 1.5 mL of MilliQ water. Strand purity was assessed using an Analytical RP-HPLC with a Phenomenex Clarity 5 μ Oligo RP LC 150 x 4.6 mm column using the same solvent system. The oligonucleotide was characterised using negative mode Electrospray Ionisation Mass Spectrometry (ES IMS). The concentration of each oligonucleotide was determined using a Shimadzu UV-1800 UV Spectrophotometer by measuring the absorbance at 260 nm.

1. J. Manchester, D. M. Bassani, J.-L. H. A. Duprey, L. Giordano, J. S. Vyle, Z.-Y. Zhao, J. H. R. Tucker, *J. Am. Chem. Soc.*, **2012**, *134*, 10791-10794.

RP-HPLC of A1

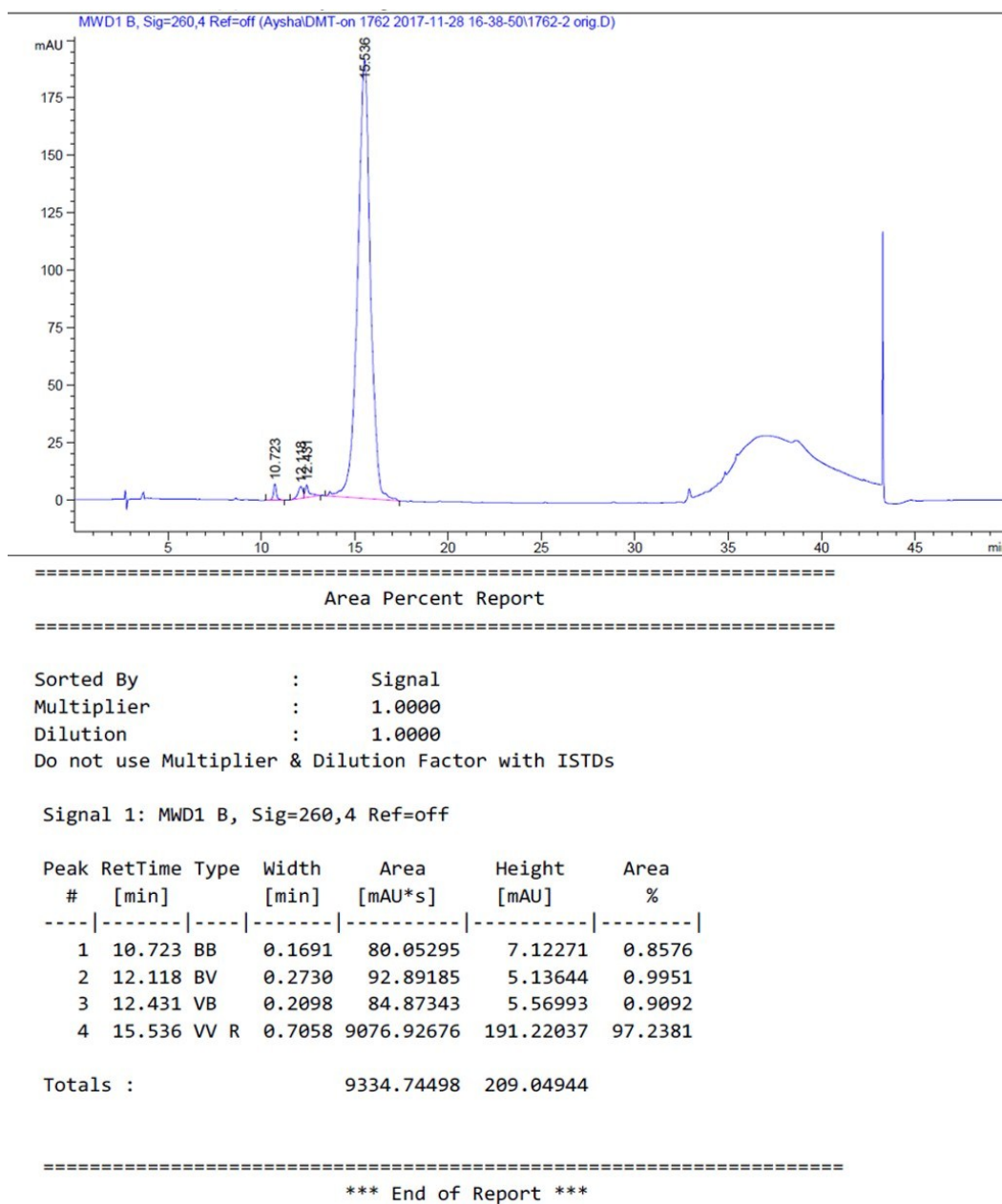
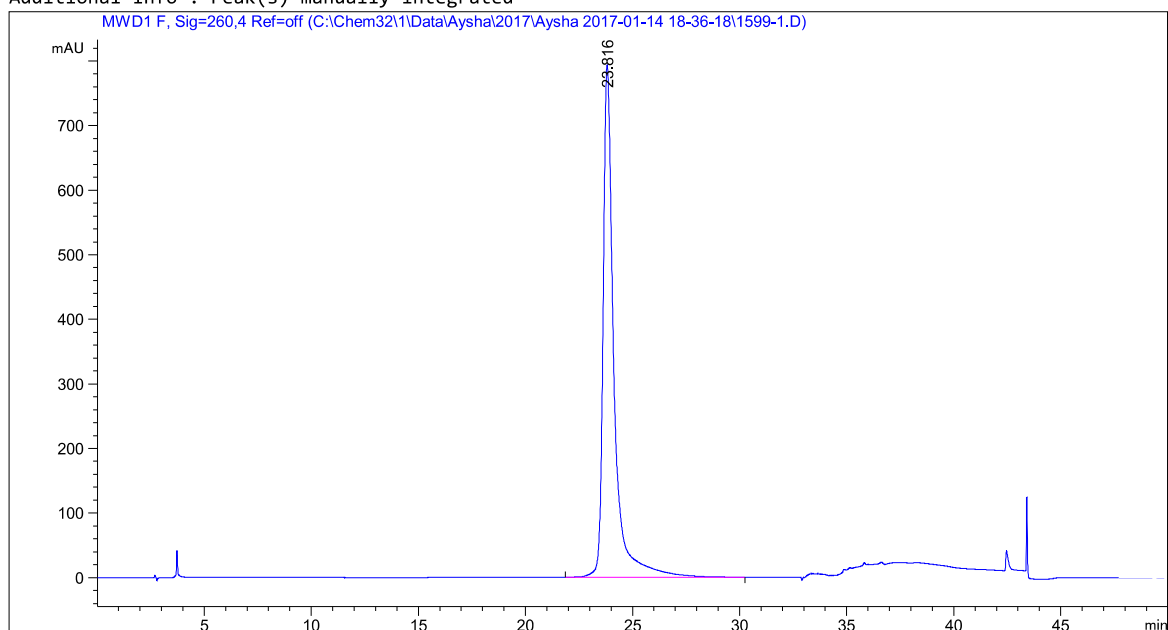


Fig S1. The analytical RP-HPLC of **A1** aptamer, showing 97% purity (eluent components at >30 minutes).

RP-HPLC of A2

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=====
Acq. Operator   : SYSTEM                      Seq. Line :    7
Acq. Instrument : LC                        Location  :    5
Injection Date  : 14-Jan-17 11:47:36 PM      Inj       :    1
                                           Inj Volume : 20.000 µl
Acq. Method     : C:\Chem32\1\Data\Aysha\Will-AD 2017-01-14 18-36-18\FcNA Anth.M
Last changed    : 14-Jan-17 6:36:18 PM by SYSTEM
Analysis Method : C:\Chem32\1\Data\Aysha\2017\Aysha 2017-01-14 18-36-18\FcNA Anth.M (Sequence
                                           Method)
Last changed    : 07-Dec-16 11:02:27 AM by SYSTEM
Sample Info     : 1599-1
=====
```

Additional Info : Peak(s) manually integrated



Area Percent Report

```
=====
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs
=====
```

Signal 1: MWD1 F, Sig=260,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	23.816	BB	0.5184	2.80315e4	793.08112	100.0000

```
Totals :                2.80315e4  793.08112
```

Fig S2. The analytical RP-HPLC data of A2 aptamer, showing 100 % purity (eluent components at >30 minutes).

Mass spectrum of A1

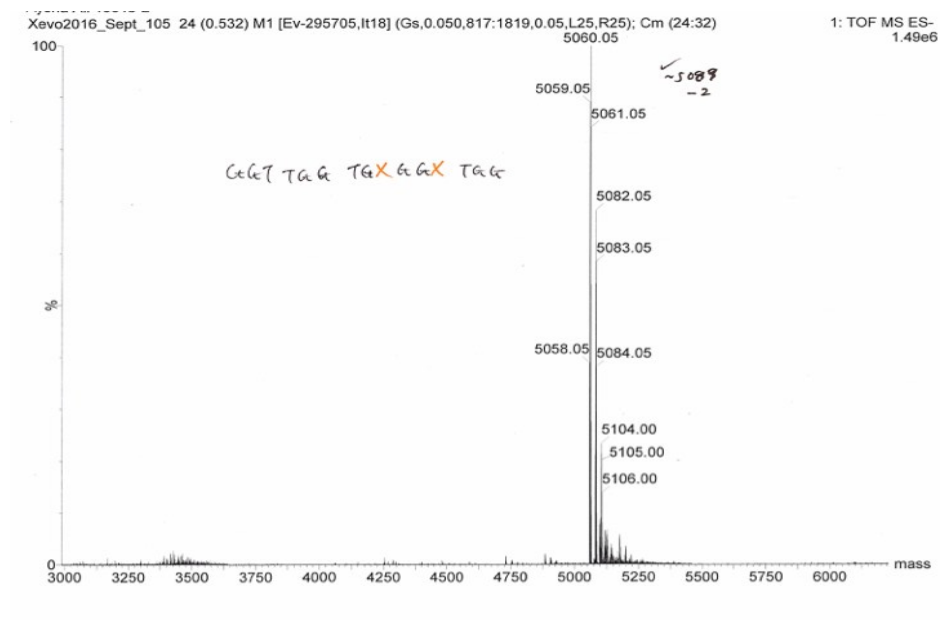


Fig S3. Electrospray ionisation mass spectrum (negative mode) of **A1** aptamer. Mass expected 5057.7, mass found 5060.05.

Mass spectrum of A2

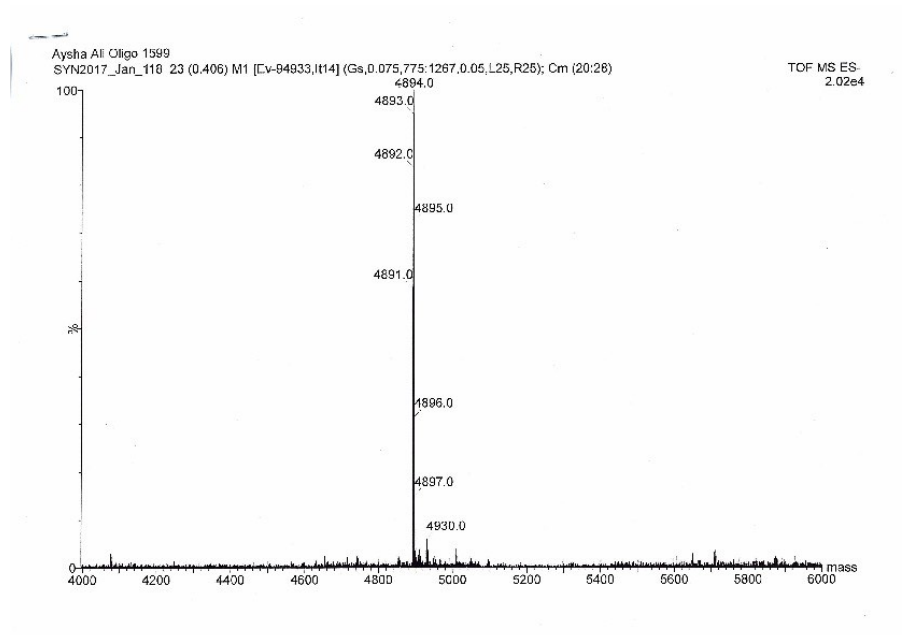


Fig S4. Electrospray ionisation mass spectrum (negative mode) of **A1** aptamer. Mass expected 4891.9, mass found 4894.0.

1.2 Photoirradiation & thermal reversion studies

The DNA strands were made up to 2.0 μM in aqueous buffer (10 mM KCl, 20 mM Tris HCl, 1 mM MgCl_2 , 120 mM NaCl, 2 mM CaCl_2 , pH 7.4). All samples were de-gassed using argon for 10 minutes before irradiation with a Hg lamp containing a 365 nm bandpass filter. Absorbance spectra recorded at hourly intervals. For the heat reversion studies, the samples were heated to 80°C for a period of 12 hours. For the **A1** sample, a 67% return to the starting material was indicated after this time, see Fig S5.



Fig S5. UV-vis spectra of **A1** before irradiation (black line), after 4 hrs photoirradiation (green line) and then after 12 hrs of subsequent heating at 80 °C (red hashed line). All samples at 2 μM in TBA buffer, RT, pH 7.4.

1.3 Circular dichroism (CD) studies

All CD studies were carried out on a Jasco J-1500 spectrometer, with thermal studies performed using a Jasco PTC-517 Peltier cell holder in the same spectrometer. A CD spectrum at 292 nm was recorded at every 5°C temperature interval from 15°C to 90 °C at a bandwidth of 5 nm.

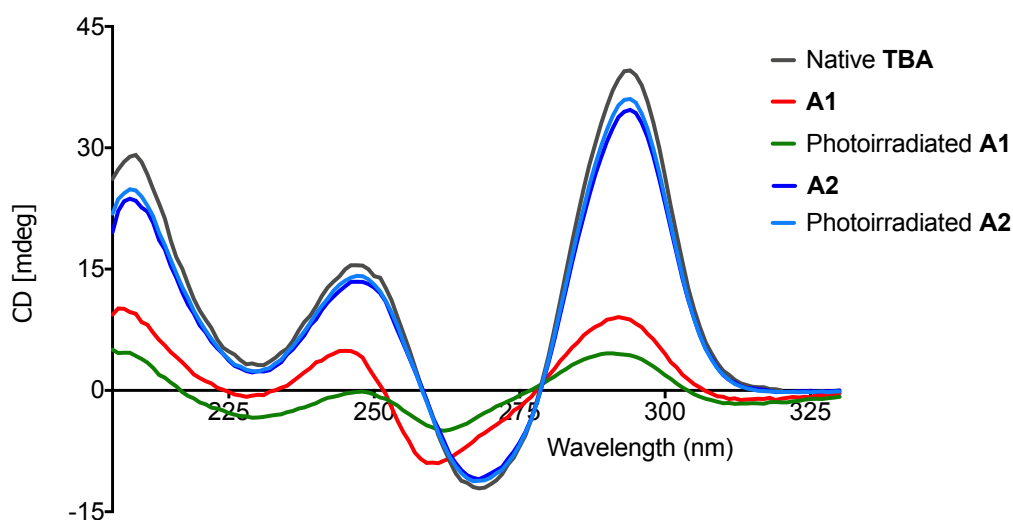


Fig S6. CD spectra of native **TBA**, **A1** both before and after 4 hrs photoirradiation and **A2**, both before and after 4 hrs photoirradiation (2 μ M in TBA buffer, pH 7.4).

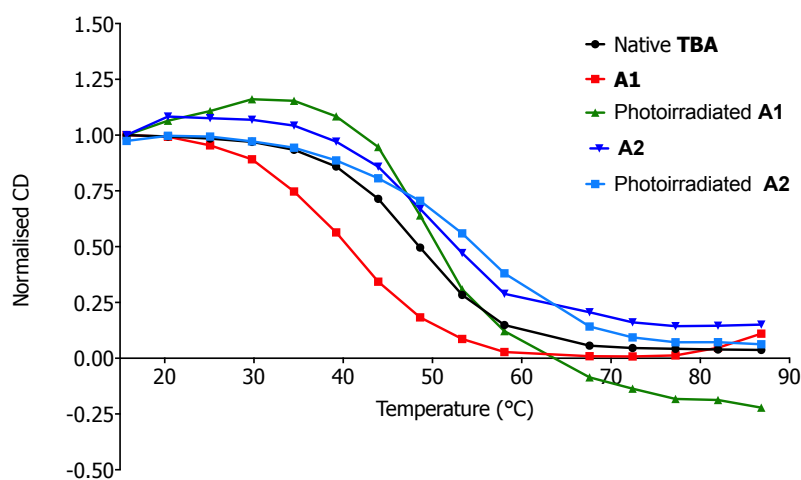


Fig S7. Thermal melt CD studies, showing the change in signal at 292 nm for **A1**, **A2** (both before and after 4 hrs photoirradiation) and **TBA** (2 μ M in TBA buffer, pH 7.4).

1.4 Electrophoretic mobility shift assay (EMSA)

EMSA studies: Gels were performed using 12% polyacrylamide gels at 30% (w/v) of Acrylamide and 0.8% (w/v) bis-acrylamide. The gel was made up to 20 mL using 10 mM KCl, 5 x TBE buffer, MilliQ water, 10% APS (150 μ L) and 15 μ L of tetramethylethylenediamine. From this, two 15 well gels were cast using glass plates with 1 mm separation. Once set, the gels were immersed in ca. 900 mL of 1 x TBE buffer + 10 mM KCl in an electrophoresis tank. Each sample was made up to 20 μ L using 2 μ L of 6X loading buffer (30% glycerol) and TBA buffer according to the concentration of thrombin and DNA required respectively.

Table S1. Concentrations of thrombin and aptamer for all EMSA studies.

Well	Thrombin (μ M)	Aptamer (μ M)
a	0.0	1.0
b	0.2	1.0
c	0.5	1.0
d	1.0	1.0
e	1.5	1.0
f	2.0	1.0
g	2.5	1.0

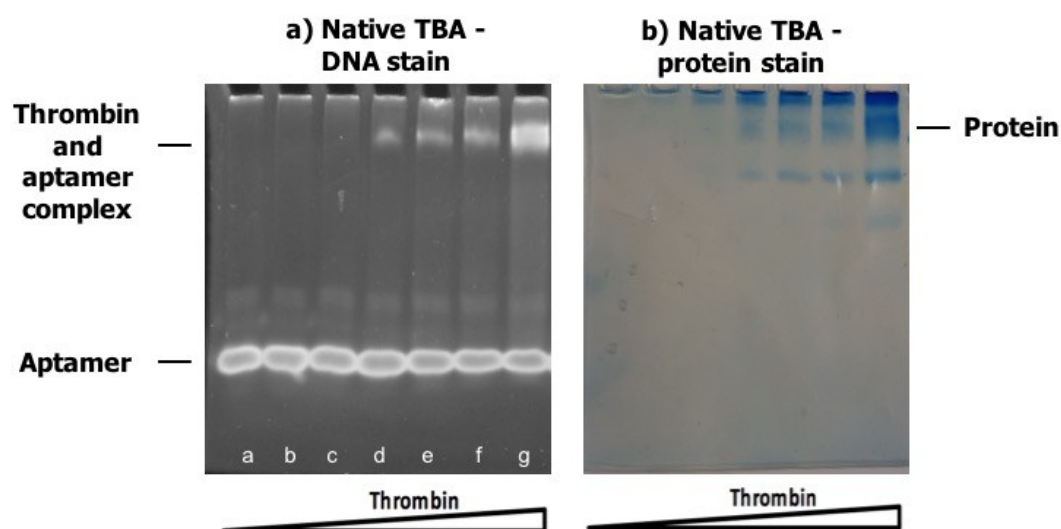


Fig S8. EMSA results of native *TBA* with thrombin. Gel a) shows the DNA stain prior to staining for protein and Gel b) shows the same gel under a protein stain. Contents of the gels can be seen in **Table S1**. All aptamers were in TBA buffer, pH 7.4.

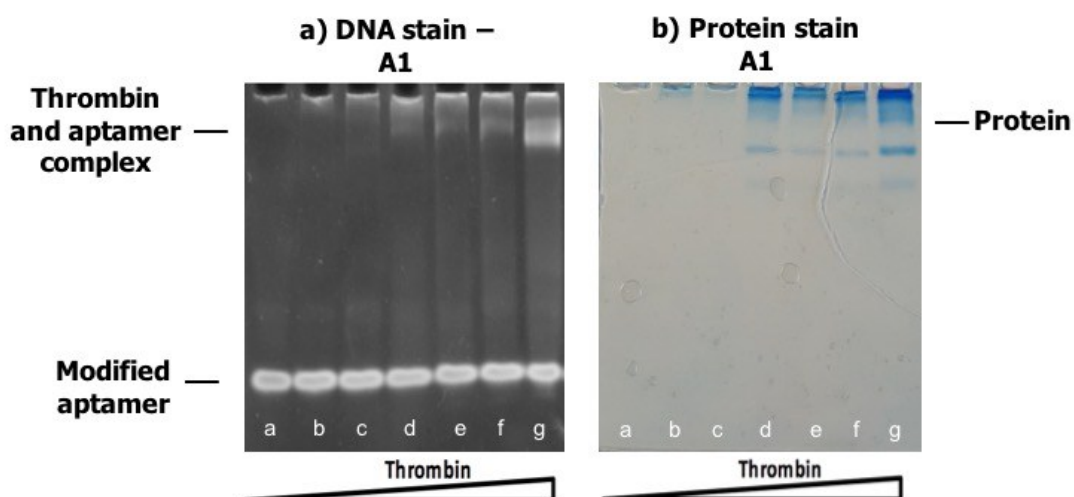


Fig S9. EMSA runs of *A1* with thrombin. Gel a) shows the DNA stain prior to staining for protein and Gel b) shows the same gel under a protein stain. Contents of the gels can be seen in **Table S1**. All aptamers were in TBA buffer, pH 7.4.

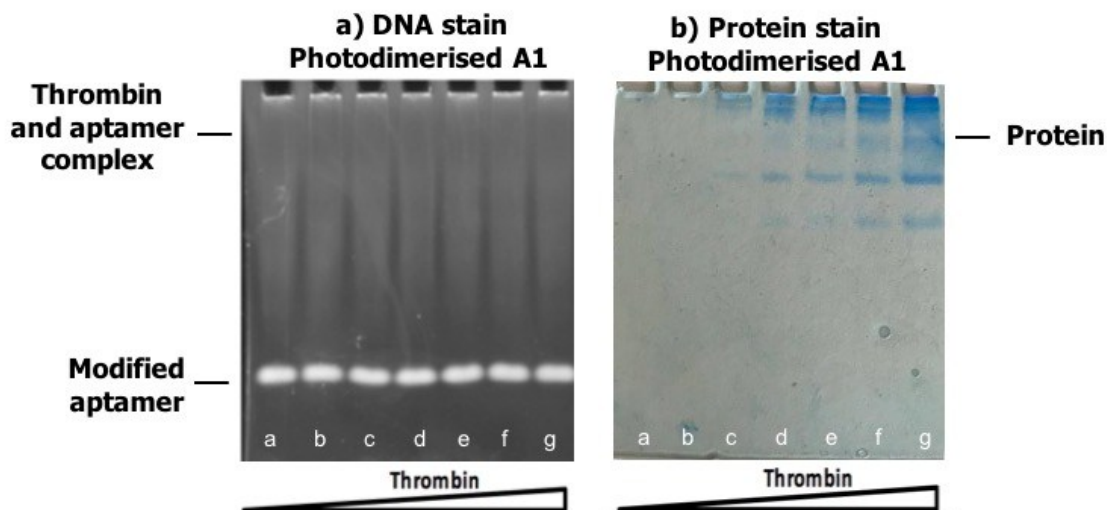


Fig S10. EMSA runs of photoirradiated **A1** (4 hrs) with thrombin. Gel a) shows the DNA stain prior to staining for protein and Gel b) shows the same gel under a protein stain. Contents of the gels can be seen in **Table S1**. All aptamers were in TBA buffer, pH 7.4.

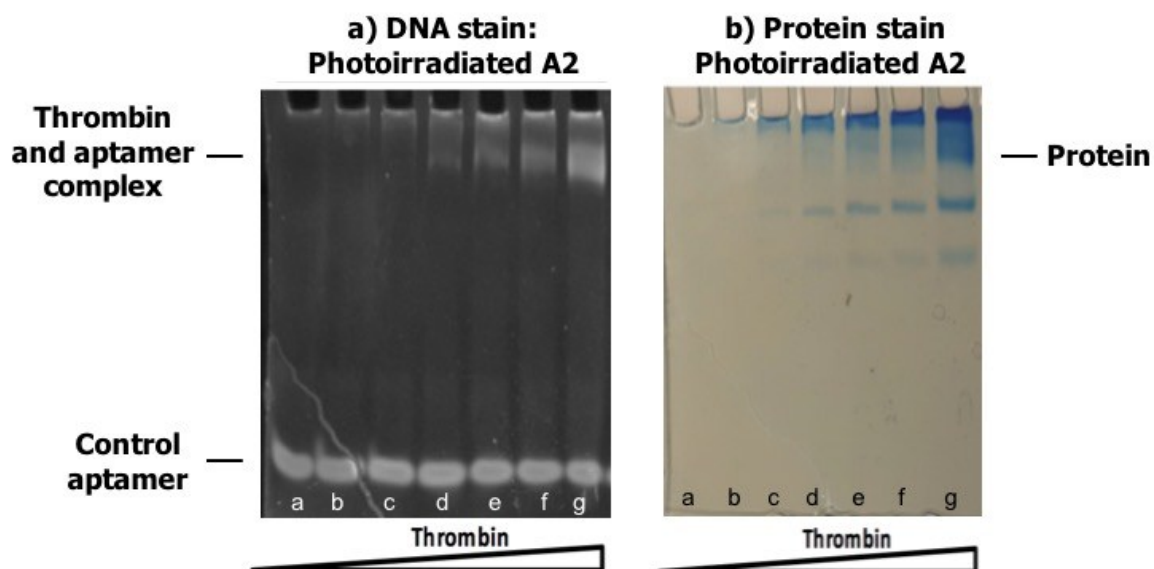


Fig S11. EMSA runs of photoirradiated **A2** (4 hrs) with thrombin. Gel a) shows the DNA stain prior to staining for protein and Gel b) shows the same gel under a protein stain. Contents of the gels can be seen in **Table S1**. All aptamers were in TBA buffer, pH 7.4.

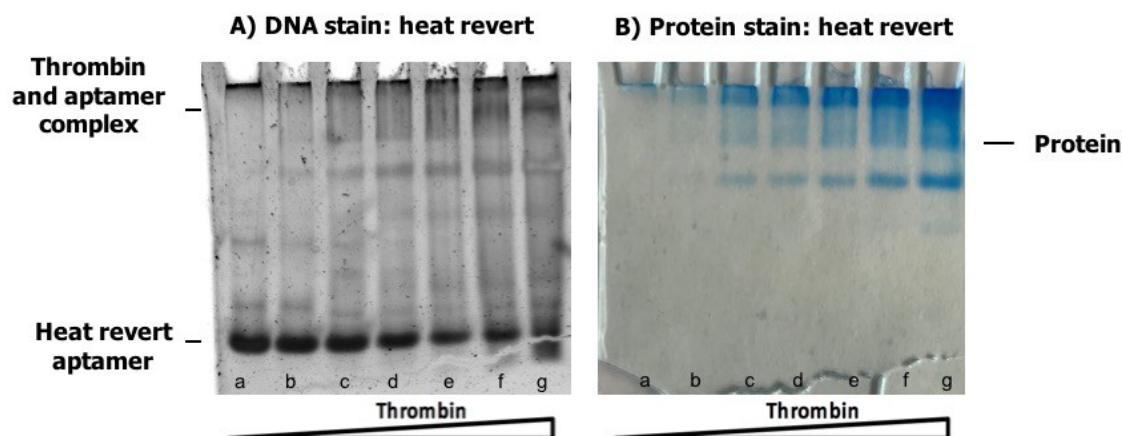


Fig S12. EMSA runs of heat reverted **A1** (12 hrs) with thrombin. Gel a) shows the DNA stain prior to staining for protein and Gel b) shows the same gel under a protein stain. Contents of the gels can be seen in **Table S1**. All aptamers were in TBA buffer, pH 7.4.

1.5 Claus Assay

As per the Helena Biosciences protocol,² thrombin (100 NIH/mL) was made up to 2 mL with MilliQ water and left for 10 minutes before use. Fibrinogen was made up to 1 mL in the buffer provided and left for 15 minutes before being diluted further with the same buffer (1:9 dilution). The aptamer samples were as used in the other studies (TBA buffer at 1 μ M). A 0.1 mL sample of the thrombin solution or a thrombin:aptamer solution (the latter at 1 μ M each and incubated for 30 minutes at 37 °C beforehand) was then added to 0.4 mL of the fibrinogen solution. The clotting reaction was monitored using the percentage change in UV-vis transmission of the sample at 450 nm over a period of 40 minutes.

2. <http://www.helena-biosciences.com/en/haemostasis/assays/routine/clauss-fibrinogen-100/>