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Supplemental information

Rapid molecular imaging of active thrombi *in vivo* using aptamer-antidote probes

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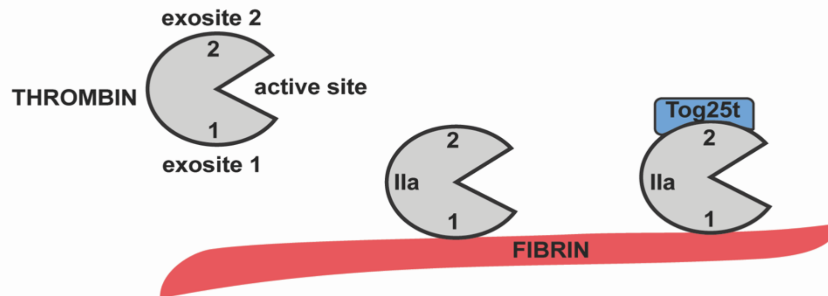
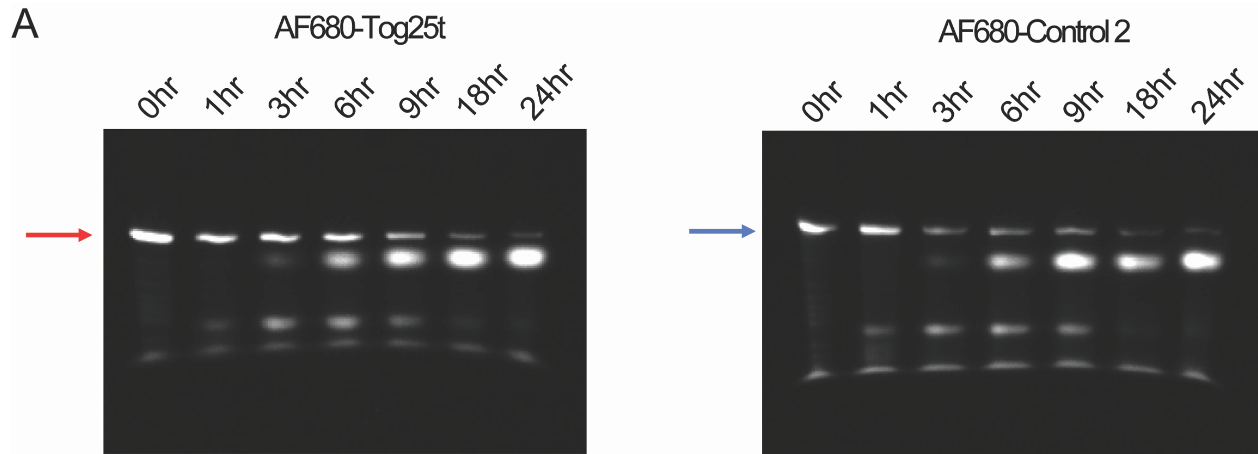
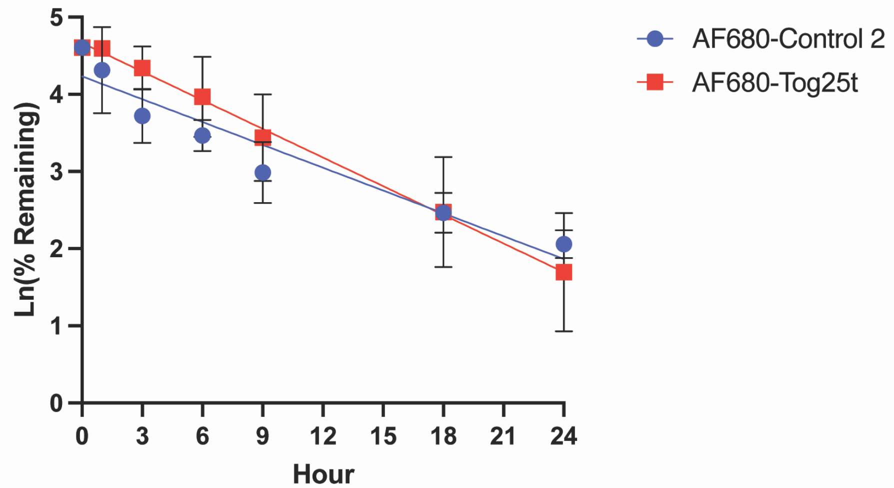


Figure S1. Schematic demonstrating binding of Tog25t to exosite 2 of thrombin. Exosite 1 of thrombin (IIa) interacts with fibrin leaving exosite 2 available for Tog25t binding.



B **Aptamer Stability in Mouse Serum**



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	Mouse Serum	
	Rate of Decay	Half Life (Hours)
AF680-Tog25t	-0.1238	5.60
AF680-Control 2	-0.09863	7.03

Figure S2. Stability of AF680-Tog25t and Control 2 in mouse serum. AF680-Tog25t or Control 2 were incubated in triplicate in mouse serum for 24hrs. At various time points, small aliquots were removed and the RNA precipitated prior to analysis via denatured polyacrylamide gels. Gels were visualized for AF680 fluorescence, and the amount of RNA remaining quantified via band intensity. *(A)* Representative gel images are shown for each conjugate in mouse serum. The red and blue arrows indicate the conjugate bands that were analyzed for the AF680-Tog25t and Control 2, respectively. *(B)* The relative band intensity based on % conjugate remaining was

graphed according to its first-order degradation to determine half-lives of the conjugates. *(C)* Calculated half-lives for each conjugate.

Video S1. Intravital microscopy reveals that Tog25t binds to pre-exisiting clots *in vivo*. Representative video from a mouse injected with fibrin antibody (anti-fibrin) via a jugular catheter before exposing a femoral vein. An electrolytic stimulus was used to injure the femoral vein to form a discrete sub-occlusive clot. Intravital imaging was begun, and 20 mins after clot initiation, 2 nmoles of AF680-Tog25t injected via the jugular catheter. The video covers from the time of clot initiation, appearing as green signal due to anti-fibrin staining, to 1 hr after clot initiation. AF680-Tog25t signal appears in red.

Video S2. Intravital microscopy reveals that Control 2 does not bind to pre-exisiting clots *in vivo*. Representative video from a mouse injected with fibrin antibody (anti-fibrin) via a jugular catheter before exposing a femoral vein. An electrolytic stimulus was used to injure the femoral vein to form a discrete sub-occlusive clot. Intravital imaging was begun, and 20 mins after clot initiation, 2 nmoles of AF680-Control 2 injected via the jugular catheter. The video covers from the time of clot initiation, appearing as green signal due to anti-fibrin staining, to 1 hr after clot initiation. AF680-Control 2 signal appears in red.

SUPPLEMENTAL MATERIALS AND METHODS

Serum Stability

All mouse work was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*[1] and approved by the Duke University Institutional Animal Care and Use Committee. Male and female C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Five mice were anesthesized with isoflurane and blood collected from the inferior vena cava. Serum was prepared using Microvette 500 z-gel tubes (Sarstedt, Numbrecht, Germany). 10µL of 10µM of the AF680-E3 or AF680-C36 conjugates were added to 90µL of fresh serum and incubated at 37°C for 24hrs. At 0, 3, 6, 9, 12 and 24 hrs, 10µL was removed from each tubes and RNA precipitated by extraction as follows: to each tube add 50µL PBS + 100µL methanol + 50µL chloroform, vortex and then add 50µL water + 50µL chloroform before vortexing and centrifuging at 500g x 20min at 4°C. After removing the aqueous phase, 50µL of each sample was flash frozen and lyophilized. The precipitated RNA was then resuspended in 10µL of PBS, denatured in 10µL of 8M urea loading dye by heating at 65°C for 5min, and then run on a 12% denaturing acrylamide gel. Gels were visualized for AF680 fluorescence on a Bio-Rad ChemicDoc XRS+ (Bio-Rad, Hercules, CA).

SUPPLEMENTAL REFERENCES

1. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, Edn. 8th. (National Academies Press, Washington, DC; 2011).