17 September 2017 : Titration of aptamer and ssDNA long trigger in lysate

1. Aim:

Titrate both aptamer and ssDNA long trigger between 10 uM and 0.5 uM in M15 T7 / Top10-GamS lysate

2. Materials:

- Nuclease-free water
- LacZalpha toehold
- ssDNA long trigger (27B)
- Aptamer trigger extension
- M15 T7 lysate
- Top10-GamS lysate
- Buffer A
- Energy solution
- Substrate (15 mg/mL)
- Plate reader

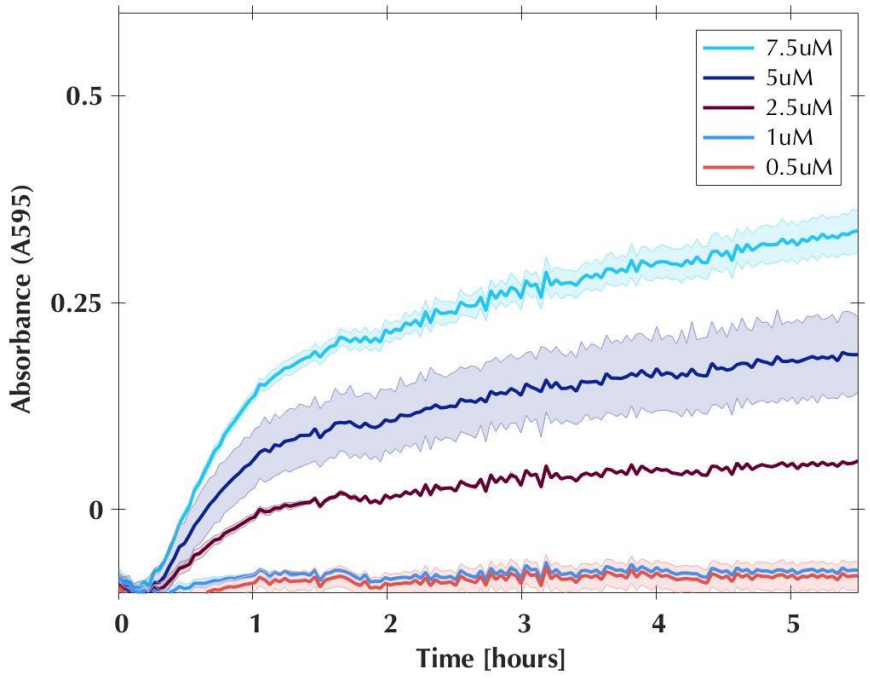
3. Procedure:

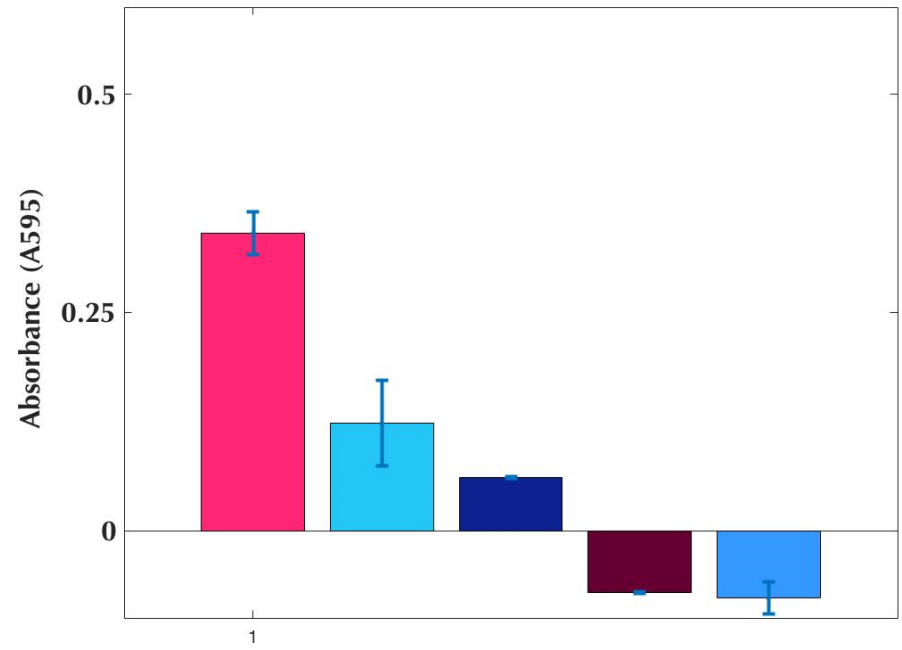
Stock trigger [uM]	final trigger [uM]	Which Trigger	Trigger quantity	[DNA] initial	DNA quantity	Which lysate	Lysate quantity	Top10-GamS lysate	Energy solution	Buffer A	Rnase inhib	H20	Substrate
500	10	aptamer ext	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2
50	7.5	aptamer ext	1.5	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	0.200	0.2
50	5	aptamer ext	1	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	0.700	0.2
50	2.5	aptamer ext	0.5	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.200	0.2
50	1	aptamer ext	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2
5	0.5	aptamer ext	1	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	0.700	0.2
		none	0	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.700	0.2
500	10	ssDNA LONG 27B	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2
50	7.5	ssDNA LONG 27B	1.5	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	0.200	0.2
50	5	ssDNA LONG 27B	1	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	0.700	0.2
50	2.5	ssDNA LONG 27B	0.5	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.200	0.2
50	1	ssDNA LONG 27B	0.2	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.500	0.2
5	0.5	ssDNA LONG 27B	1	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	0.700	0.2

		NONE	0	LacZalpha Toehold	0.6	M15-T7	1.25	1.25	2.5	2.5	0	1.700	0.2

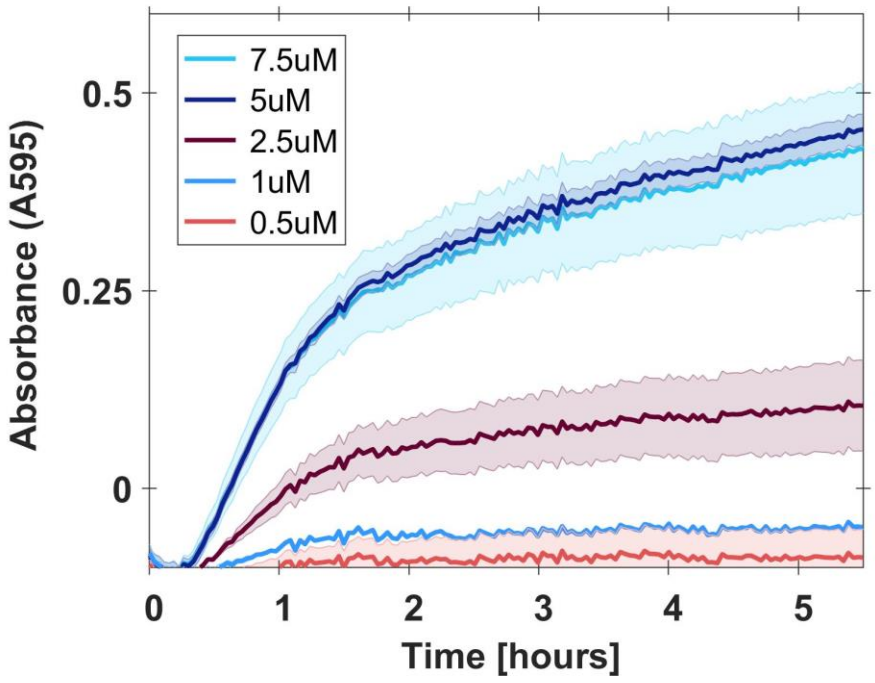
4. Results:

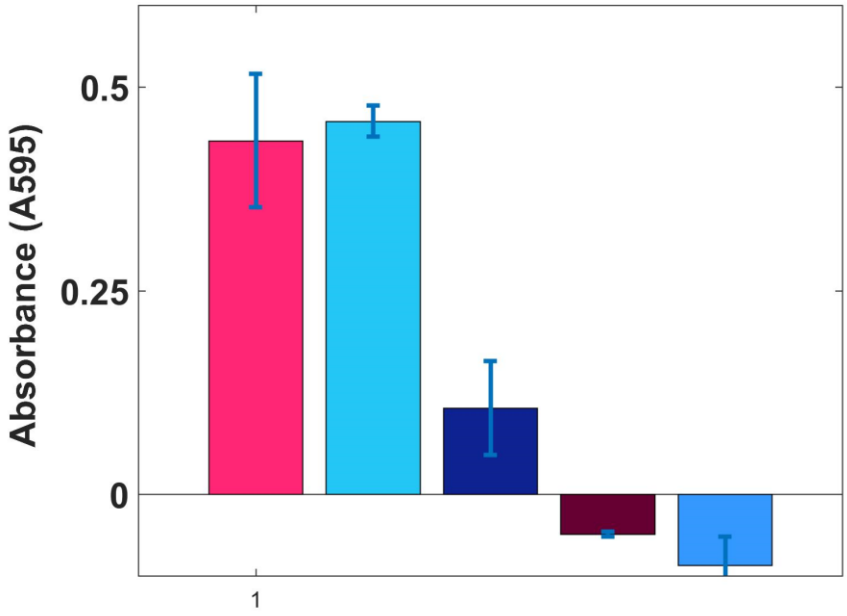
Aptamer titration : blank was subtracted





ssDNA long titration : blank was subtracted





End point measurements were taken after 6 hours.

5. Conclusion:

The ssDNA long trigger seems to be more effective at triggering the toehold. This could be due to the additional base pairs of the aptamer, which might lead to a certain steric hindrance while binding to the toehold.

Signals could be detected at 2.5 uM of trigger concentration, but not below (same for both triggers).