Supporting information

Adapting Enzyme-Free DNA Circuits to the Detection of Loop-Mediated Isothermal Amplification Reactions

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Content:

1. Supporting Figures and Tables

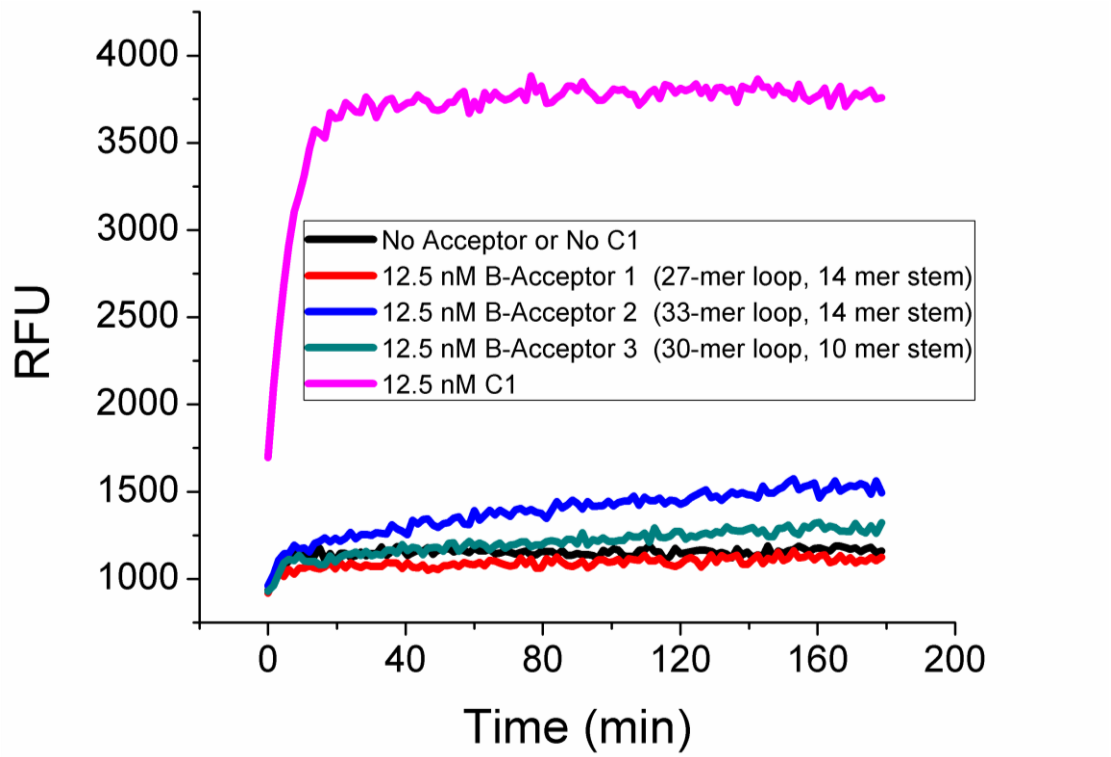

Figure S1: CHA catalyzed by **C1** (3-2-1) or by **B-acceptors** that probe **B-target**. The final concentrations of reaction components were as follows: [**C1**] = [**B-acceptors**] = 12.5 nM, [**H1**] = 50 nM, [**H2**] = 400 nM, [**F**] = 50 nM, [**Q**] = 100 nM.

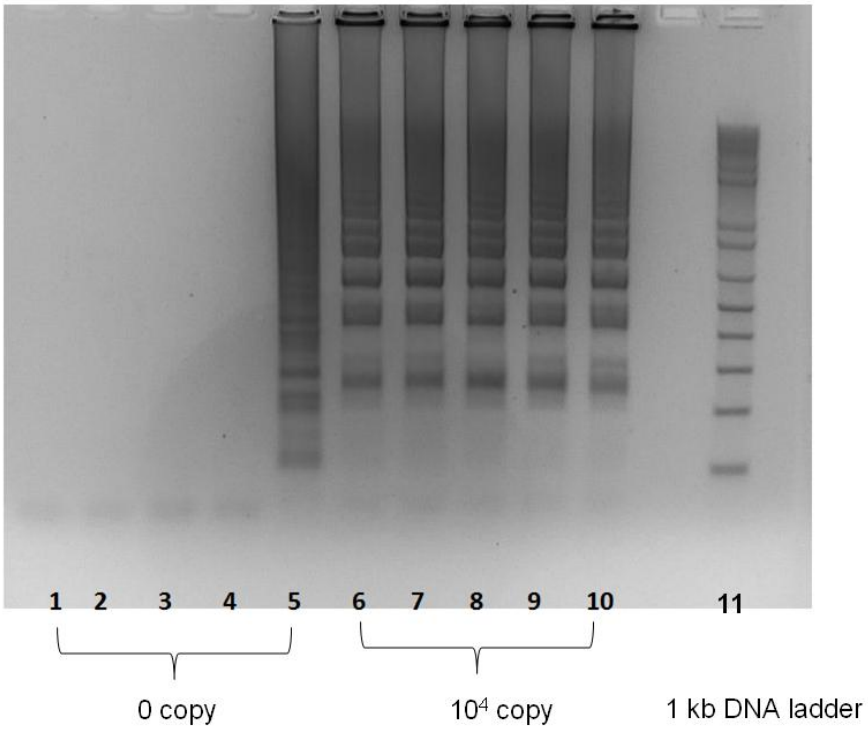

Figure S2: LAMP side reactions. Lane **1** to **5**: five parallel reactions carried out without any M13mp18 templates. Lane **6** to **10**: five parallel reactions seeded with 10^4 copies of M13mp18. Lane **11:** 1 kb Plus DNA ladder (Invitrogen). Lane 5 is indicative of the large DNA products that can sometimes arise even in primer-only reactions.

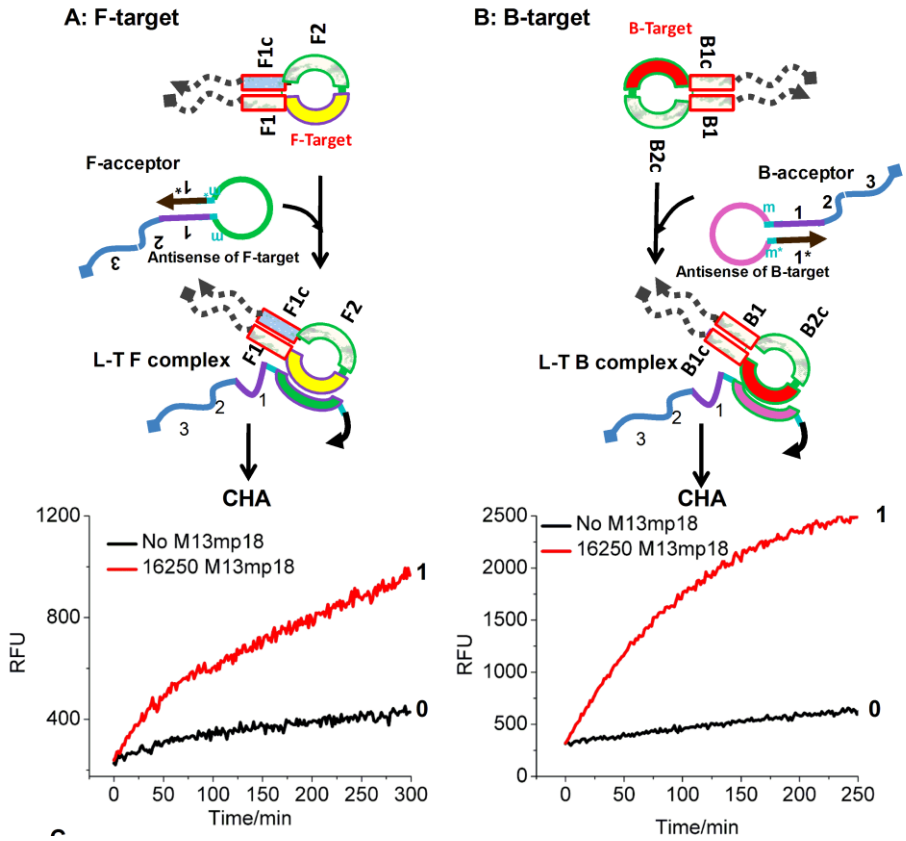

Figure S3. Coupling LAMP to a parallel CHA detector. (A) Timecourse of CHA-mediated detection of LAMP via the **F-acceptor1**. (B) Time-course of CHA-mediated detection of LAMP via the **B-acceptor1**. [**B-acceptor1**]=[**F-acceptor1**]=12.5 nM, [**H1**]=[**H3**]=50 nM, [**H2**]=[**H4**]=400 nM, [**F**]=[**F2**]=1/2[**Q**]=1/2[**Q2**]=50 nM.

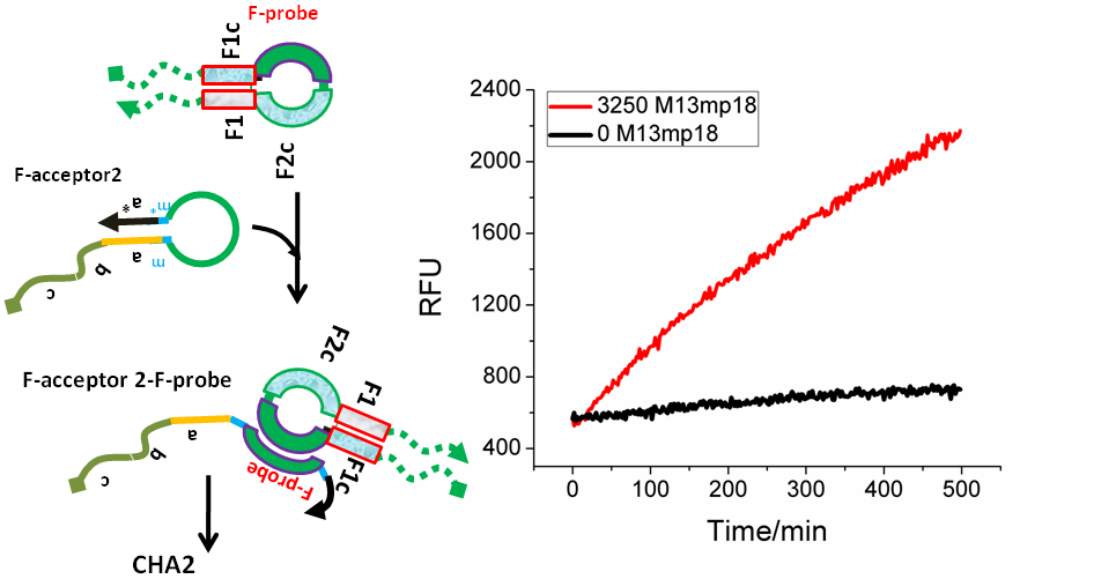

Figure S4. Probing LAMP amplicons by using **F-acceptor2** to monitor the **F-probe,** followed by the CHA2 detector. [**F-acceptor2**]=12.5 nM, [**H3**]=50 nM, [**H4**]=400 nM, [**F2**]=1/2[**Q2**]=50 nM.

Table S1: Oligonucleotides used in this work.

Name		Sequence (5'-3')	Modification
M13mp18 primer set	**B3**	GTTGGGAAGGGCGATCG	
	B1c-B2	ACAACGTCGTGACTGGGAAAACCCTTTTTGTGCGGGCCTCTTC GCTATTAC	
	F3	ACTTTATGCTTCCGGCTCGTA	
	F1c-F2	CGACTCTAGAGGATCCCCGGGTACTTTTTGTTGTGTGGAATTG TGAGCGGAT	
B-acceptors with loop sequences complementary to **B-target**	**B-acceptor 1**	CGAC**ATCT**AACCTAGC*TCACTGAC*TTAAATGTGCTGCAAGGCG ATTAAGTTGGGTTTAAGTCAGTGAGC	Note: The underlined sequence is the loop sequence, and the italicized sequence is the toehold for the CHA catalyst
	B-acceptor 2	CGACATCTAACCTAGC*TCACTGAC*TTAACGTTACCCAACT TAATCGCCTTGCAGCACATCCTTAAGTCAGTGAGC	
	B-acceptor 3	CGACATCTAACCTAGC*TCACTGAC*TTAAATGTGCTGCAAGGCG ATTAAGTTGGGTAACTTAAGTCAGGAGC	
F-acceptor with loop sequences complementary to **F-target**	**F-acceptor 1**	CGACATCTAACCTAGC*TCACTGAC*CGATATTCGTAATCATGGT CATAGCTGTTATCG GTCAGTGAGC	
F-acceptor with loop sequences complementary to **F-probe**	**F-acceptor 2**	GAAATGGCCGAAGATGCTC*TAAAGTGC*AAACAGCTATGACCAT GATTACGAATGCACTTTAGA	
CHA1 reaction set	**H1**	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATC TAACCTAGCCCTTGTCATAGAGCAC	
	H2	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGT AGA	
	F in reporter1	CGAGTGCTCTATGACAAGGGCTAGGTT	5' FAM
	Q in reporter1	CCCTTGTCATAGAGCACTCG	3' IowaBlack FQ
	C1 =3-2-1	CGACATCTAACCTAGCTCACTGAC	
CHA2 reaction set	**H3**	GCACTTTAGAGCATCTTCGGCCATTTCGCTATATCCTCCACGG AAATGGCCGAAGATGCTCCTGATGTGGGCTAAAG	
	H4	GCCATTTCCGTGGAGGATATAGCGAAATGGCCGAAGATGCTC GCTATATCCTCCACG	
	F2 in reporter2	GCTAGGCTTTAGCCCACATCAGGAGCATCTTCG	5' FAM
	Q2 in reporter2	CCTGATGTGGGCTAAAGCCTAGC	3' IowaBlack FQ
	C2=c-b-a	GAAATGGCCGAAGATGCTCTAAAGTGC	
AND gate reporter set	**F3**	CGAGTGCTCTATGACAAGGGCTAGGTCTTTAGCCCACATCAG GAGCATCTTCG	5' FAM
	Q in reporter1	CCCTTGTCATAGAGCACTCG	3' IowaBlack FQ
	M	CCTGATGTGGGCTAAAGACCTAGC	
MB used in control experiment to molecular beacon		CACTGAC ATGTGCTGCAAGGCGATTAAGTTGGGT GTCAGTG	3' IowaBlack FQ 5'FAM

2. A note on differential loop concentrations

There are 4 loops that transiently form during the production of LAMP products: **F-target**, **B-probe**, **B-target**, and **F-probe** (as shown in **Figure 1**). However, the average lifetimes of these loops are different. Loops **F-target** and **B-probe** are created during a strand displacement reaction. The completion of this displacement reaction results in a hairpin loop that can be extended by the polymerase. This extension will turn loops **F-target** and **B-probe** into duplexes. Since unimolecular folding and primer extension are relatively fast reactions, loops **F-target** and **B-probe** are quickly extinguished.

In contrast, loops **B-target** and **F-probe** are inactivated by a bimolecular primer-binding event followed by primer extension. At standard primer concentrations, the relative rate of primer-binding should be lower than for unimolecular folding, and the lifetimes of these loops are correspondingly longer than those of **F-target** and **B-probe**.