Supplementary Materials

Methods

RecA was purchased from New England Biolabs. Each RecA protein sample purchased was tested against previous samples to ensure consistent performance between batches. Oligonucleotides were purchased from IDT DNA (sequences in Table S1). Hairpin components and C90 were PAGE-purified using standard techniques. Hairpin components were annealed fresh for each reaction by heating at 95°C for 5 min, then cooling to room temperature at a rate of 0.1°/sec. CHA reactions were performed similarly to previously published methods[1, 2] with modifications to accommodate RecA; briefly, reactions were assembled in the presence or absence of 2.5 nM catalyst (for positive and negative reactions), 2 mM ATP, 4 mM $MgCl_2$ in TNaK buffer (20 mM Tris pH 7.5, 140 mM NaCl, 5 mM KCl, 1 μM dA-dT dsDNA blocker) with 50 nM H1, 400 nM H2, and 50 nM reporter complex with concentrations of RecA as indicated. H1, H2, and the Reporter components RepF and RepQ were identical in sequence to previous publications[2]; C30 is identical to a 24 nt catalyst used in former studies[2] with the exception that the sequence GTG was added at both the 3' and 5' ends. C90 is identical to C30, except that 30 nt of additional GTG repeats are added to each end. Where indicated, annealing reactions were performed before CHA assembly using 10 nM single-stranded catalyst and 1 μM RecA in the presence of 2 mM dATP and RecA buffer (New England Biolabs, 70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, pH 7.6) at 37°C for 10 min unless otherwise indicated. Annealing reactions were diluted into the CHA reactions to a final catalyst concentration of 2.5 nM. For Figure 2, reactions were initiated with the addition of H1, as was done previously[2]. All other experiments were initiated by the addition of catalyst or pre-annealed RecA-catalyst complex, as this produced the most consistent results, especially in the case of pre-annealed complexes.

Kinetic data from fluorescent reporter molecules was collected using a Tecan infinite M200 Pro plate reader set for 490 nm excitation and 525 nm emission collection, with reads every minute for 45 minutes. Fluorescence values were normalized for each experiment, with 0 representing the minimum fluorescence value and 100 representing the maximum fluorescence value for each individual experiment, unless otherwise noted. Kinetic rates were determined by taking the slope of each normalized kinetic plot between 10 and 20 min of a 45 min run. This was generally determined to be the linear portion of the reaction. Bar graphs represent the average of three experimental replicates; error bars are the standard error of the mean.

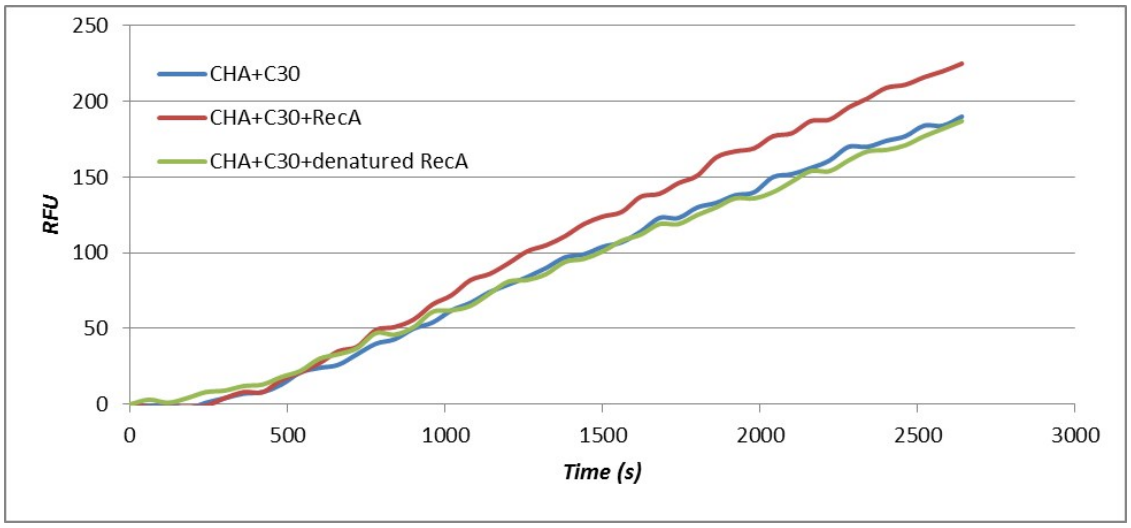

Figure S1. Fluorescent signal of CHA circuits and catalyst (C30) with RecA and denatured RecA over time. RecA was included at a concentration of 250nM. RecA was denatured at 65°C for 20 min where applicable. All curves were normalized to an initial read of 0. The rate of the circuit (slope of the line) increases when RecA is added (red compared to blue), but does not increase when denatured RecA is added (green).

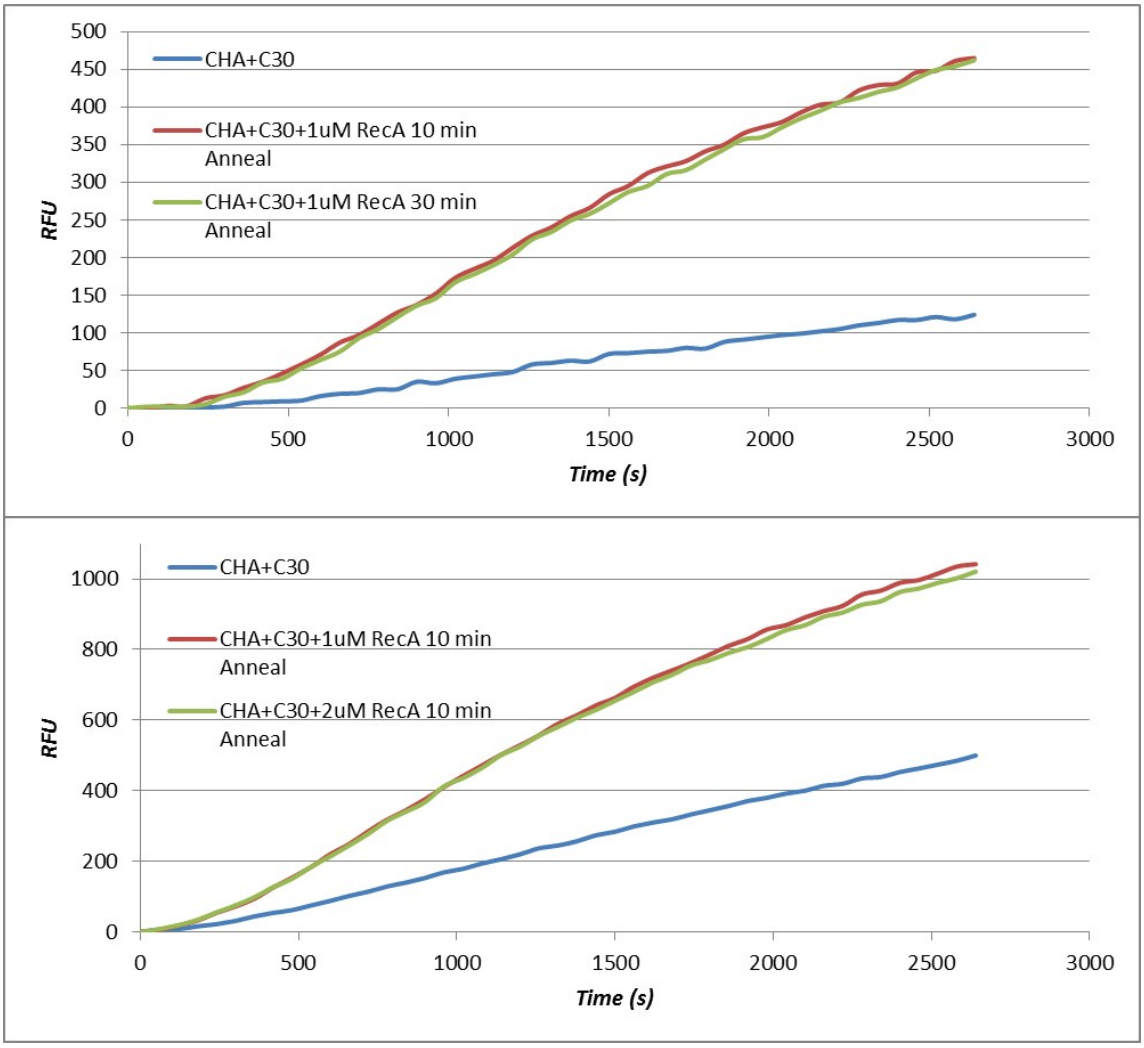

Figure S2. Fluorescent signal of CHA circuits and catalyst (C30) with RecA+C30 annealing reactions carried out before CHA analysis as indicated. Annealing reactions were carried out at 37°C; similar results were seen at 17°C (data not shown). All curves normalized to an initial read of 0. Longer anneal times did not change reaction rates (red compared to green, top). Increasing [RecA] during annealing also did not change reaction rates (red compared to green, bottom).

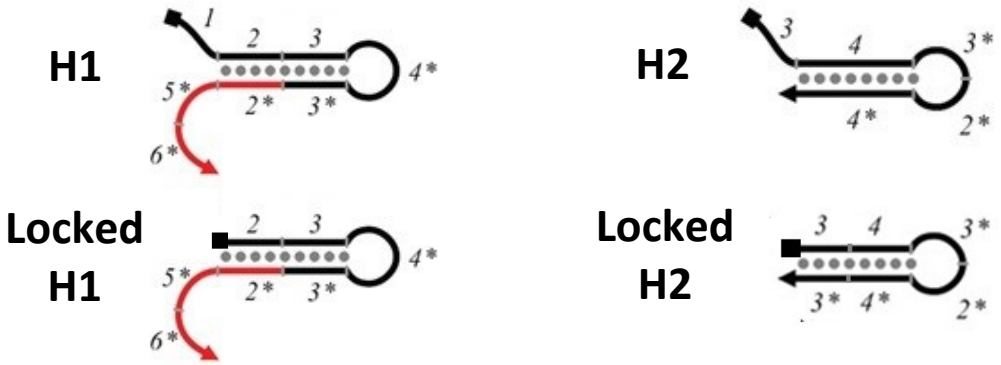

Figure S3. Design of "locked" CHA hairpins to isolate background reactions. Squares indicate 5' end, arrows indicate 3' end. Red indicates sequence which triggers the reporter (see Figure 1). For each hairpin, the toehold was either removed (H1) or complemented (H2) to prevent regular CHA reactions and isolate background reactions specifically when either is used; locked H1 will not open in the presence of catalyst, while locked H2 will not open and regenerate catalyst in the presence of opened H1. Originally, H1 was also complimented by changing the 1 domain to a 5 domain as was done with H2; this requires a change in catalyst sequence, however, so we ultimately chose to present this approach. In subsequent locked hairpin experiments (Figure S4), this did not change the data (not shown).

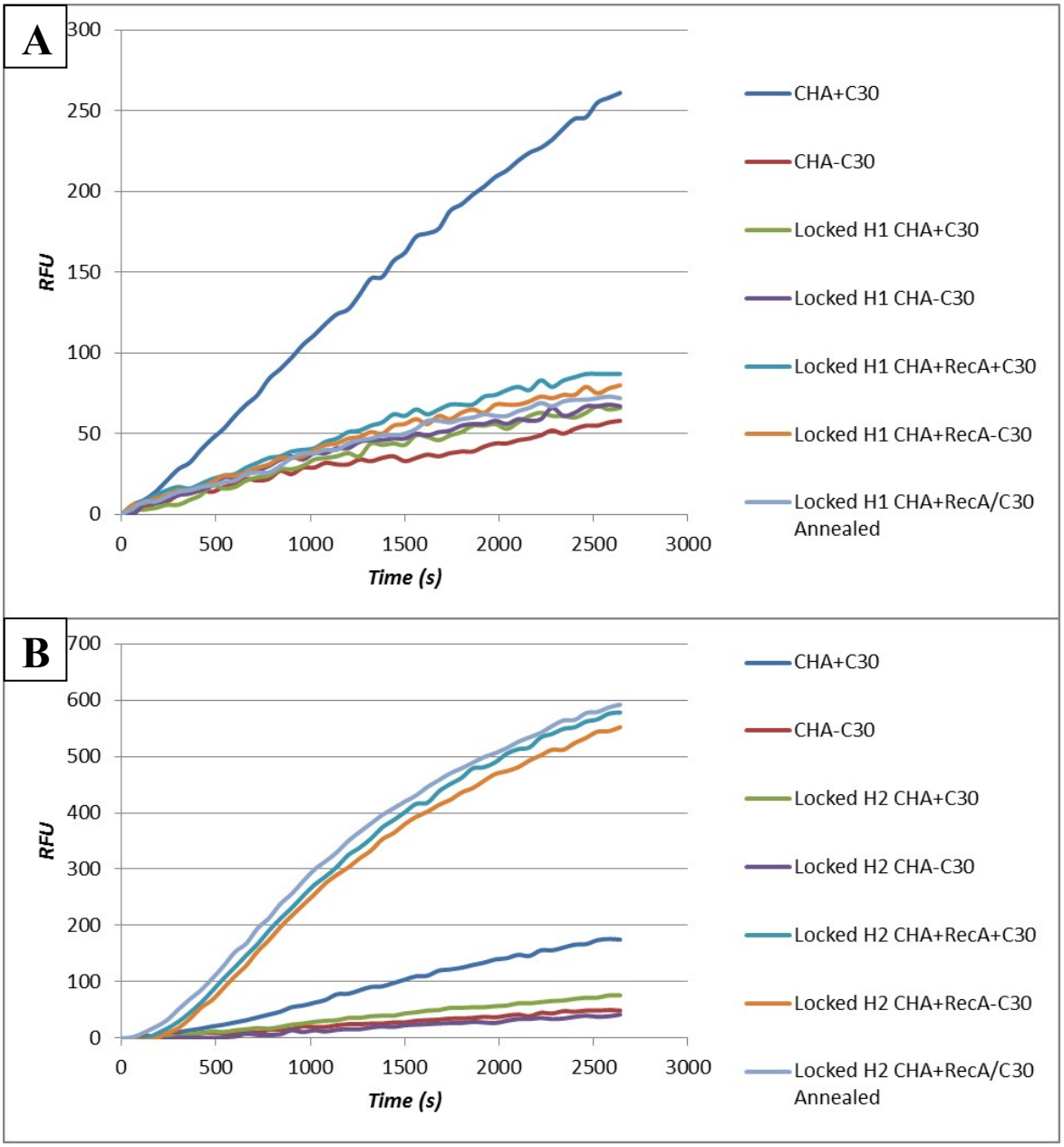

Figure S4. Fluorescent signal of CHA circuits and catalyst (C30) with "locked" H1 (A) and "locked" H2 (B, see Figure S3). All curves were normalized to an initial read of 0. For locked H1, signal stays low regardless of the addition of RecA or annealed RecA/C30, indicating that background reactions remain low. With locked H2, signal remains low in the absence of RecA, but becomes as high as other RecA CHA reactions with the addition of RecA, regardless of the addition of catalyst. Furthermore, these reactions indicate that the rates are the same regardless of annealing or presence of catalyst. This indicates significant background levels, leading us to believe that the loop of H2 was responsible for our high background in RecA CHA circuits.

C30	GTG CGA CAT CTA ACC TAG CTC ACT GAC GTG
C90	GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG CGA CAT CTA ACC TAG CTC ACT GAC GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
H1	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGCCCTTGTCATAGAGCAC
H2	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGTAGA
RepF	/56-FAM/CGAGTGCTCTATGACAAGGGCTAGGTT
RepQ	CCCTTGTCATAGAGCACTCG/3IABkFQ/
Locked H1	GCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGCCCTTGTCATAGAGCAC
Locked H2	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGTAGACGACATCT
RNA Loop H2	AGATGTCGTCTACACATGGCGACAUCUAACCUAGCCCATGTGTAGA

Table S1 Oligonucleotide sequences for all components used in the experiment are indicated. The catalyst region of the two catalyst sequences are indicated in brown. The RNA loop of the chimeric H2 used in Figure 5 are indicated in red. The FAM and Iowa Black labels of the reporter are indicated in IDT format.

References

1. Y. S. Jiang, B. Li, J. N. Milligan, S. Bhadra and A. D. Ellington, *Journal of the American Chemical Society*, 2013, **135**, 7430-7433.
2. B. Li, A. D. Ellington and X. Chen, *Nucleic acids research*, 2011, **39**, e110.