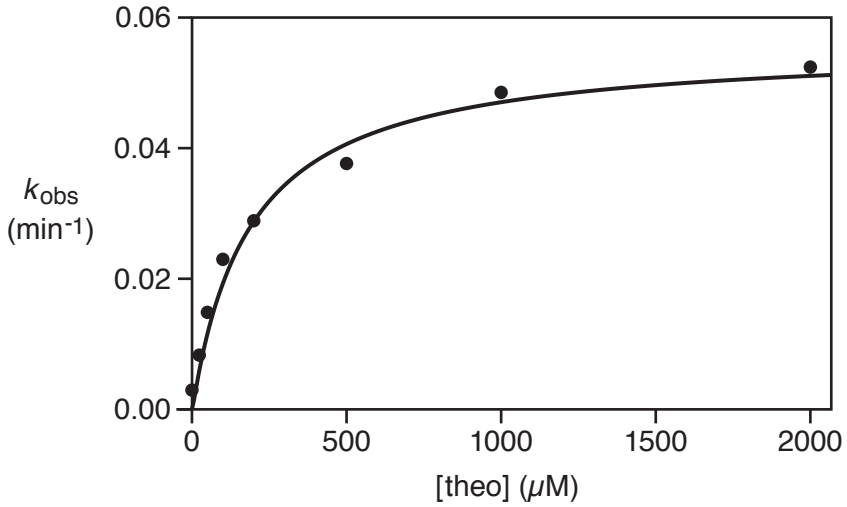
Supporting Information

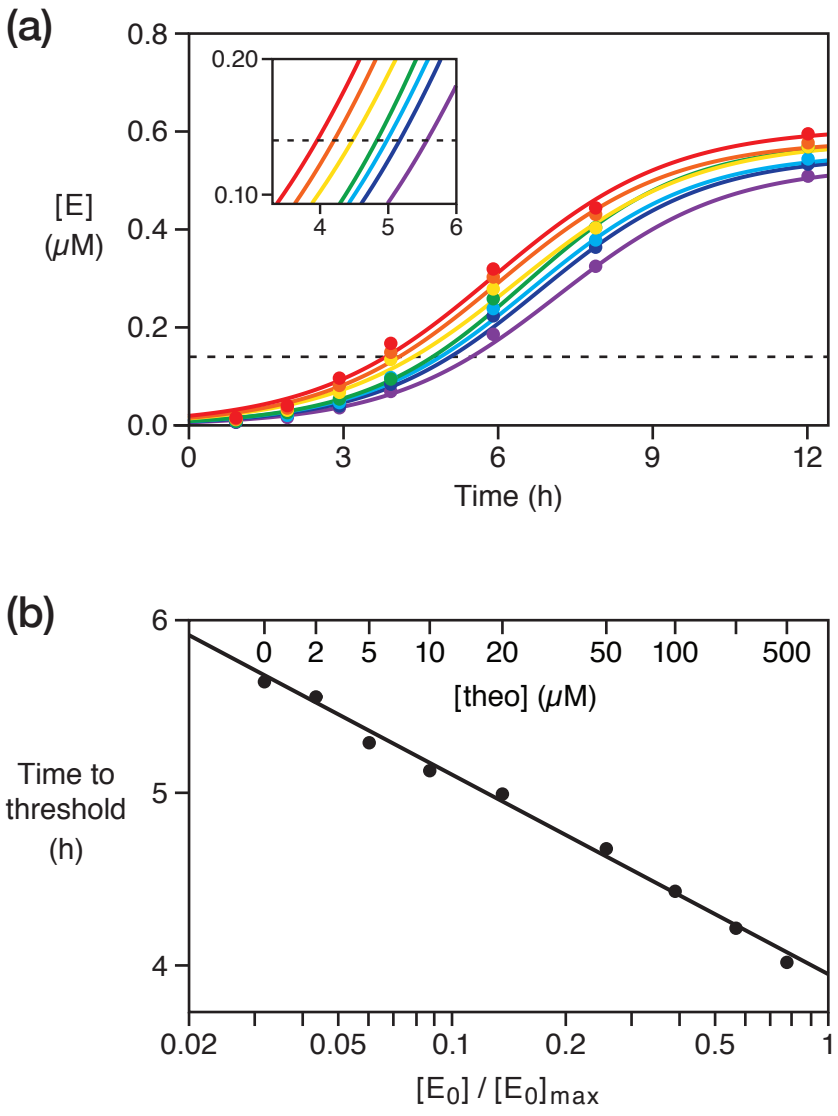
Supplementary Figure 1. Observed rate of E_{theo} as a function of theophylline concentration. For each theophylline concentration, the reaction was sampled at 0, 5, 10, 20, 30, 60, 120, and 180 min, the fraction reacted was determined by PAGE, and these data were fit to the equation: $F_t = F_{max} (1 - e^{k_{obs} \cdot t})$, where F_t is the fraction reacted at time t and F_{max} is the maximum extent. Values for k_{obs} were fit to a saturation plot: $k_{obs} = k_{cat}$ [theo] / (K_d + [theo]), which gave a k_{cat} of 0.055 min^{-1} and K_d of 160 μM (r = 0.993). Reaction conditions: 5 μM E_{theo}, 0.5 μM each of A_1 and A_2-B, 25 mM $MgCl_2$, pH 8.5, 42 °C.

Supplementary Figure 2. Quantitative, isothermal, ligand-dependent exponential amplification in the presence of 10% deproteinized serum. (a) The yield of E (and E′) was monitored over time in the presence of 0 (purple), 5 (blue), 10 (cyan), 20 (green), 50 (yellow), 100 (orange), or 200 (red) μM theophylline. These data were fit to the equation: [E] = a / (1 + $b e^{-c t}$), where a is the final extent, b is the degree of sigmoidicity, and c is the exponential growth rate. A threshold (dashed line) was set at 0.14 μM E, corresponding to 25% of the maximum extent of amplification. Inset shows each amplification profile as it crossed the threshold. Amplification profiles for 2 and 500 μM theophylline are omitted for clarity. (b) Time to threshold as a function of the concentration of E_0, which reflects the concentration of theophylline. Linear regression coefficient was 0.997.

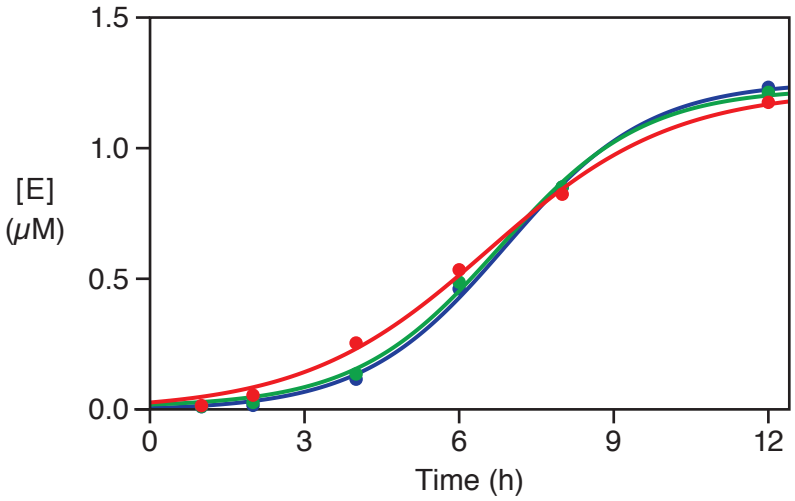
Supplementary Figure 3. Exponential amplification initiated by E_0 in a reaction mixture that also contained: 5 μM E_{theo} and no theophylline (blue), 5 μM E_{theo} and 1 mM theophylline (green), or 0.5 μM each of A_1 and A_2-B and no theophylline (red). Reaction conditions: 2 μM each of A, A′, B, and B′, 25 mM $MgCl_2$, pH 8.5, 42 °C.

Supplementary Figure 1




(a)
[E]
(μM)
0.8
0.6
0.4
0.2
0.0
0.20
0.10
4
5
6
0
3
6
9
12
Time (h)
(b)
Time to
threshold
(h)
6
5
4
0
2
5
10
20
50
100
500
[theo] (μM)
0.02
0.05
0.1
0.2
0.5
1
[E0] / [E0]max

Supplementary Figure 3



Supplementary Table 1. Spontaneous Initiation of Cross-replication by A, B, A´, and B´ [a]

A•A´ pairing	[A], [B], [A´], [B´] (μM)	exp. growth rate (h^{-1})	maximum extent (μM)	time to threshold [b] (h)
GAAU•GUUU	5 [c]	1.17	3.29	1.96
GAU•GUU	5 [c]	0.68	3.04	4.18
GAU•GUU	5	0.77	3.22	3.27
GAU•GUU	4	0.62	2.76	3.63
GAU•GUU	3	0.56	2.09	4.24
GAU•GUU	2	0.52	1.34	5.52
GAU•GUU	1	0.45	0.62	8.61

[a] Reaction conditions: 25 mM $MgCl_2$, pH 8.5, 42 °C. [b] Time to threshold is the time required to reach 25% of maximum extent. [c] B and B´ substrates were prepared using the standard T7 promoter and did not contain a self-cleaving hammerhead ribozyme at the 3´ end.