Supplementary Material for

Characterizing and Prototyping Genetic Networks with Cell-Free Transcription-Translation Reactions

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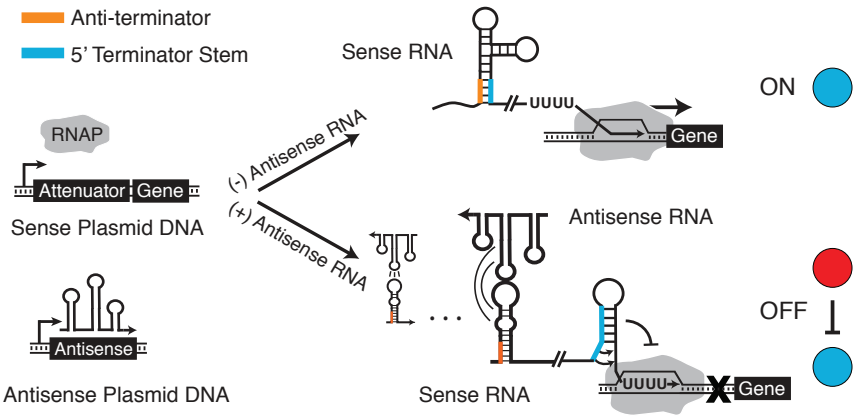

Figure S1. The transcriptional attenuation mechanism from the *Staphylococcus aureus* plasmid pT181 [1,2]. The attenuator lies in the 5' untranslated region of the transcript and can fold into a structure that will allow transcription to continue if antisense RNA is not present (ON). Antisense RNA binding to the attenuator causes the formation of a transcription terminator hairpin, stopping transcription before the gene of interest (OFF, indicated by x symbol). Colored circles represent an abstract view of the switch. The antisense RNA (red circle) represses (blunt end line) the attenuator-reporter gene target (blue circle).

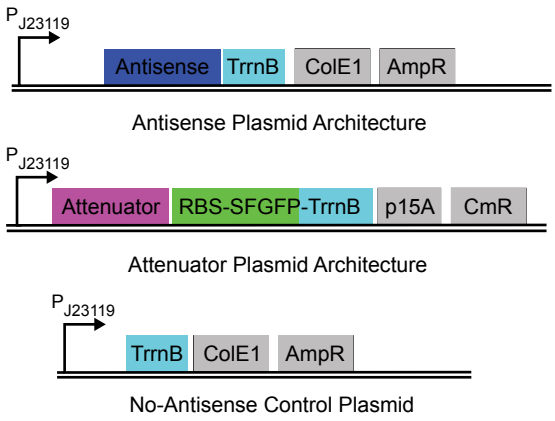

Figure S2. Plasmid architecture for attenuator and antisense plasmids. Antisense plasmids have the ColE1 origin and ampicillin resistance (AmpR). Attenuator plasmids have the p15A origin and chloramphenicol resistance (CmR). The J23119 *E. coli* consensus promoter (http://partsregistry.org/Part:BBa_J23119), modified to include a SpeI site right before the start of transcription, was used for all plasmids. TrrnB is a transcriptional terminator. RBS = ribosome binding site; SFGFP = super folder green fluorescent protein coding sequence.

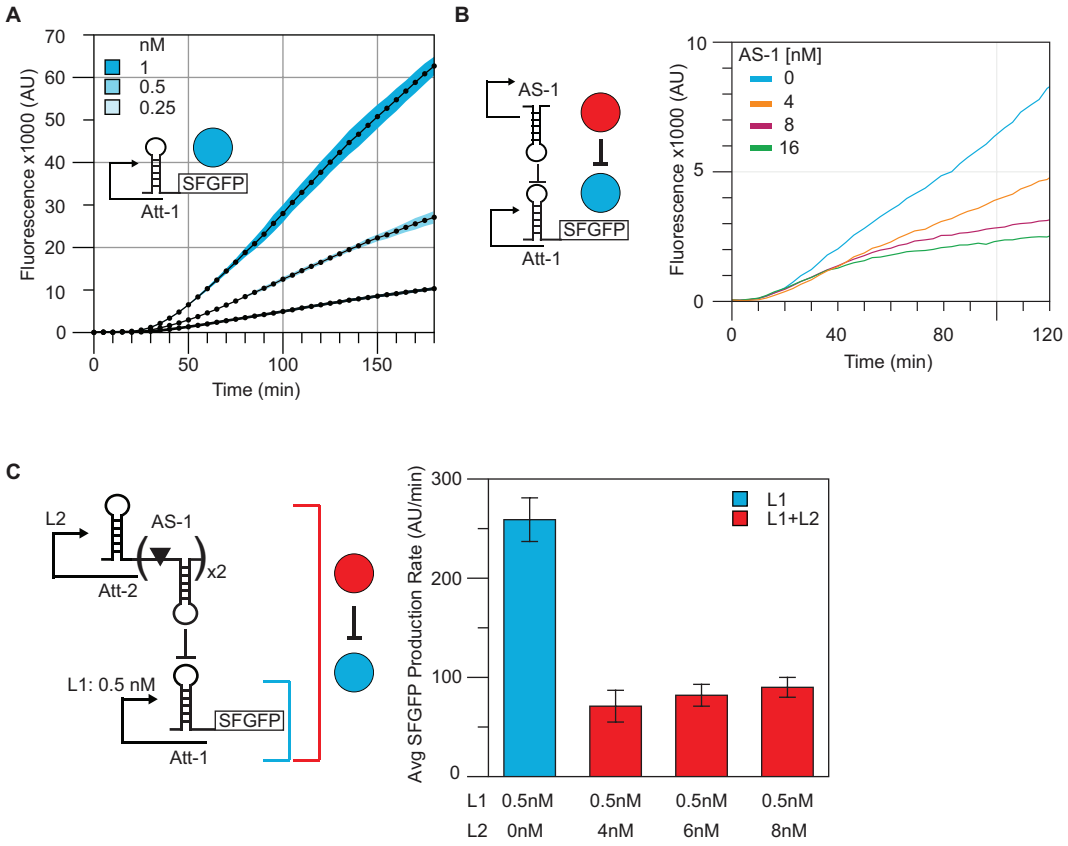

Figure S3. Titration data for the RNA network example in Figure 2. (A) Average fluorescence time courses of TX-TL reactions containing the pT181 attenuator plasmid with varying (0.25 – 1nM) concentrations. Shaded regions represent standard deviations of four independent reactions calculated at each time point. (B) Schematic of repression of the pT181 attenuator (Att-1) by its antisense RNA (AS-1). Fluorescence time courses for TX-TL reactions with 0.5 nM attenuator plasmid and varying antisense RNA concentrations (0 – 16nM). Traces represent a single TX-TL reaction collected on a different plate reader, thus a different fluorescence output than A, C, and Figure 2. (C) Schematic of the first two levels of the cascade in Figure 2. L1 is the same pT181 attenuator (Att-1) reporter plasmid used in Figure 2 (A) – (D). In the plasmid for L2, the pT181-mut attenuator (Att-2) controls transcription of two copies of the pT181 antisense (AS-1), each separated by a ribozyme (triangle). Average SFGFP production rates for TX-TL reactions with 0.5 nM L1 and varying concentrations of L2 (4 – 8nM). Error bars represent standard deviations from four independent reactions. Figure adapted from Takahashi et al., ACS Synth. Biol., 4 (2015) 503-515 [3].

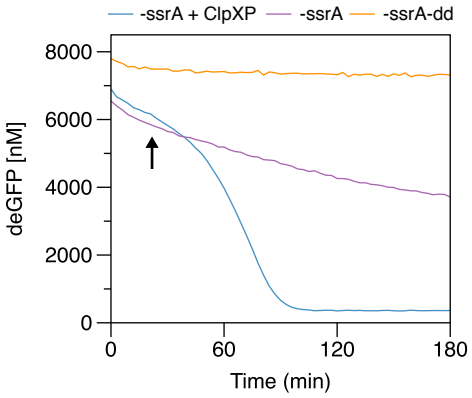

Figure S4. Protein degradation by ClpXP. ClpXP selectively degrades –ssrA tagged proteins in TX-TL reactions. 4.5μM of either –ssrA or defectively –ssrA (-ssrA-dd) tagged, purified deGFP is added to a TX-TL reaction either supplemented with nothing or with 1nM of a strong-expressing plasmid expressing ClpX and ClpP. The black arrow indicates the point at which expressed ClpX and ClpP start to become active.

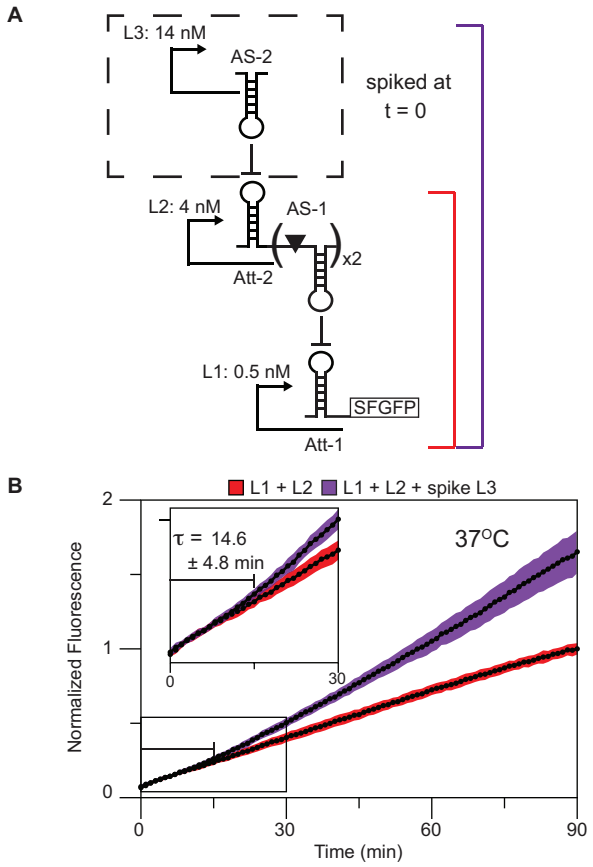

Figure S5. Determining cascade response time. (A) Schematic of a spike experiment. L3 (or the no-antisense control plasmid) was spiked into an ongoing L1+L2 TX-TL reaction at time, t = 0 (represented by dashed box). Concentrations of DNA used are indicated beside the levels. (B) Normalized fluorescence curves combining three separate experiments performed at 37 °C with a total of 11 replicates over multiple days. An L1 (0.5 nM) + L2 (4 nM) reaction was setup for 20 min at which point L3 (14 nM, puple curve) or no-antisense control DNA (14 nM, red curve) was spiked into the reaction and time reset to 0. Inset shows the response time of the circuit to the addition of L3; defined as the time at which the L3 spike curve is statistically different from the L1+L2 curve (τ = 14.6 ± 4.8 min). Figure adapted from Takahashi et al., ACS Synth. Biol., 4 (2015) 503-515 [3].

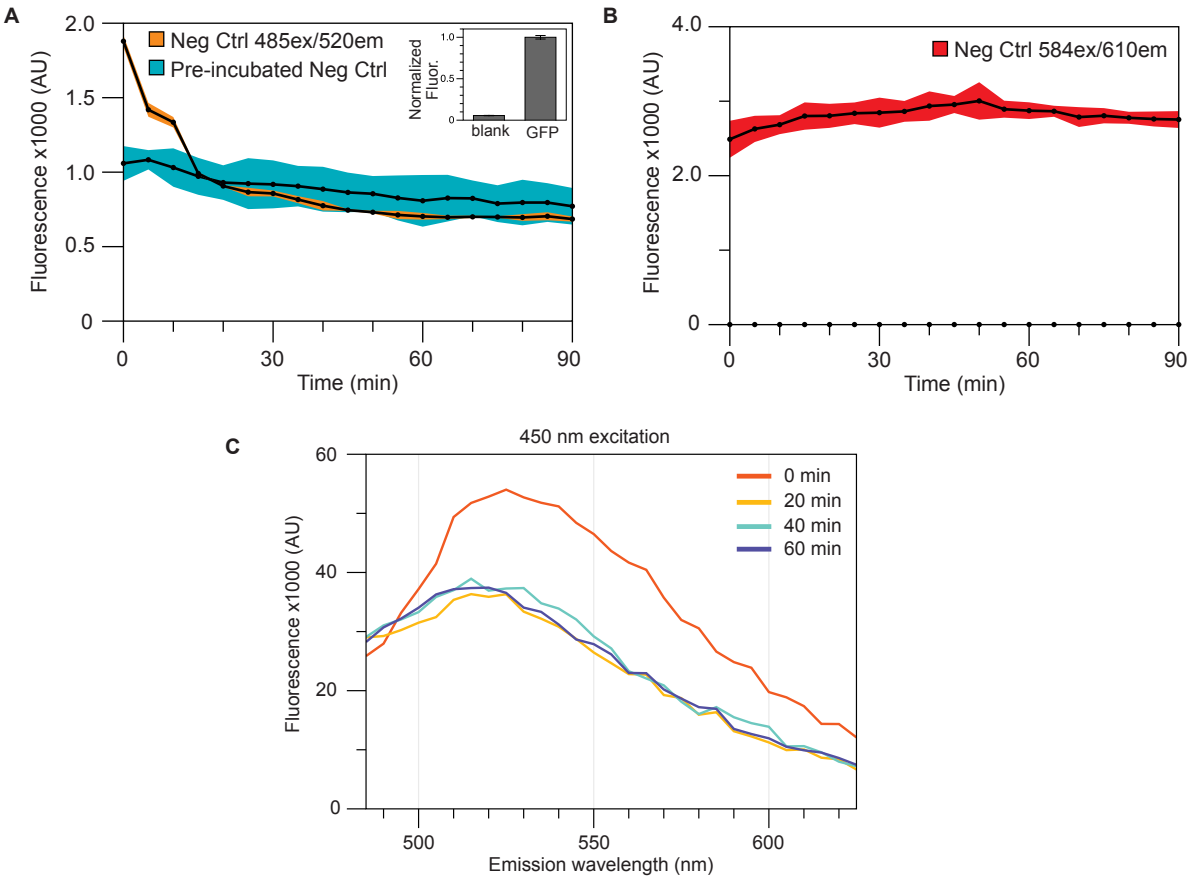

Figure S6. Fluorescence of TX-TL reaction negative control. (A) Fluorescence trajectories of the negative control with (blue) and without (orange) pre-incubation measured at 485 nm excitation and 520 nm emission. A pre-incubation of the buffer and extract for 20 min at 37^{O}C eliminates the decrease in fluorescence seen over the first 20-40 min of the reaction. Inset shows the fluorescence of the negative control relative to SFGFP fluorescence from the Att-1 construct in Figure 2A after a 2 hour reaction. Error bars represent standard deviations from three independent reactions. (B) Fluorescence trajectory of the negative control without pre-incubation measured at 584 nm excitation and 610 nm emission. Shaded regions represent standard deviations from three independent reactions. (C) TX-TL buffer and extract were mixed, added to a 384-well plate, and incubated at 37^{O}C. Emission spectra were collected (450 nm excitation) at 0, 20, 40, and 60 min after mixing.

References

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[2] S. Brantl, E.G. Wagner, Antisense RNA-mediated transcriptional attenuation: an in vitro study of plasmid pT181, Mol Microbiol. 35 (2000) 1469–1482.

[3] M.K. Takahashi, J. Chappell, C.A. Hayes, Z.Z. Sun, J. Kim, V. Singhal, et al., Rapidly Characterizing the Fast Dynamics of RNA Genetic Circuitry with Cell-Free Transcription-Translation (TX-TL) Systems, ACS Synth. Biol. 4 (2015) 503-515. doi:10.1021/sb400206c.