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Supplemental Information

Biophysical Constraints Arising from Compositional Context in Synthetic Gene Networks

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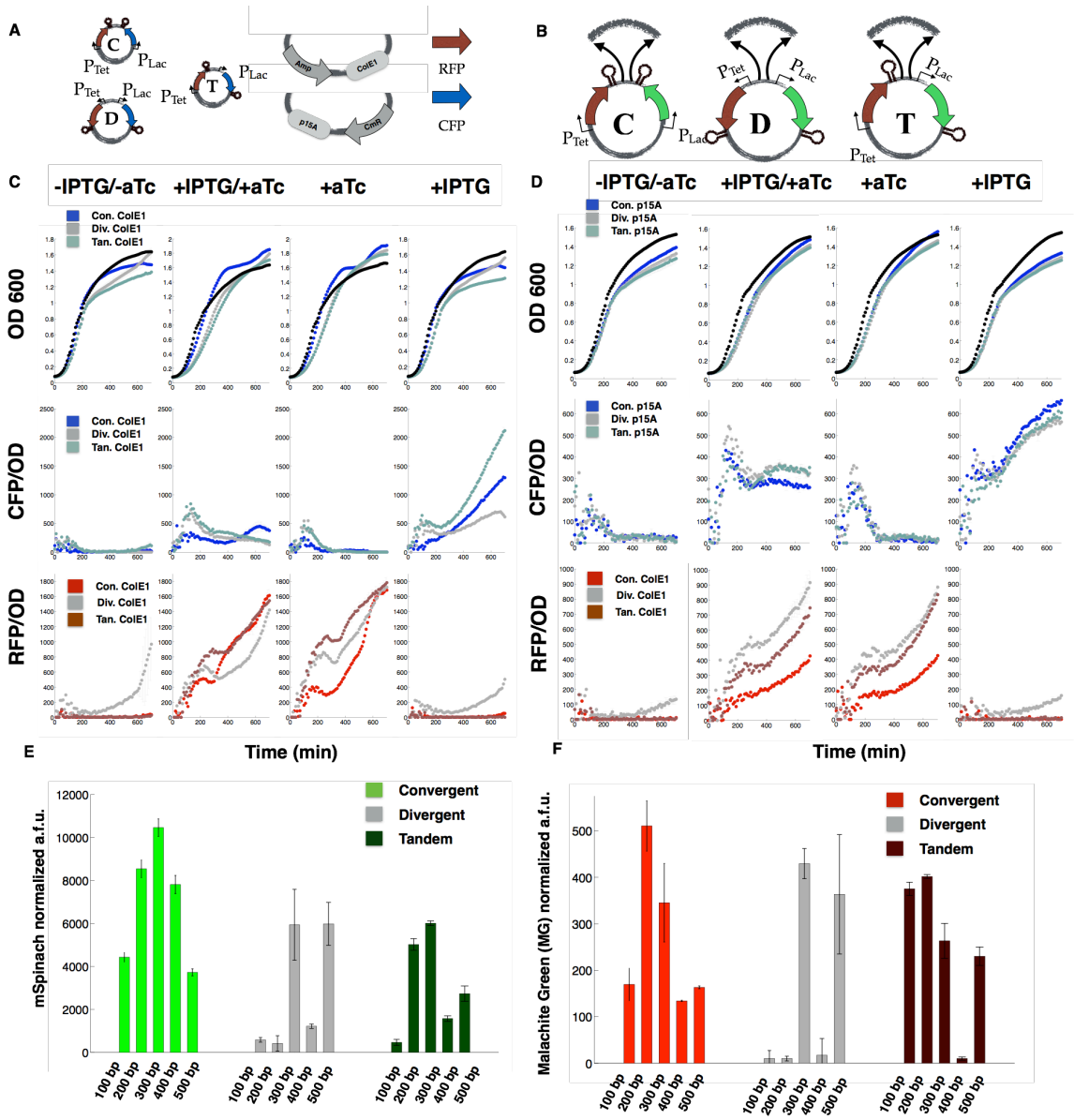

Figure S1: Related to Figures 1, 2, and 3. **(A)** (Left) Plasmid layouts for RFP and CFP in convergent, divergent, and tandem orientation, (Right) the composition of the plasmid backbone for the ColE1 and p15A backbones used in collecting data for **C-D**. **(B)** A diagram showing the sense and anti-sense CFP and RFP single gene cassette controls, expressed on the ColE1 backbone. **(C-D)** Time lapse *in vivo* plate reader expression of RFP and CFP and growth curves, induced with either 1 mM IPTG, 200 ng/mL aTc, or both, on either ColE1 plasmid or p15A plasmid backbone. **(E)** A schematic showing the point of insertion of intergenic spacing sequences of length n = 100, 200, 300, 400, and 500 bp. **(F)** Steady-state *in vivo* expression of mSpinach from overnight induction in 1 mM IPTG and 200 ng/mL aTc in convergent, divergent, and tandem orientation, varied as a function of spacer length. **(G)** Steady-state expression of MG RNA aptamer from overnight induction in 1 mM IPTG and 200 ng/mL aTc in convergent, divergent, and tandem orientation, varied as a function of spacer length.

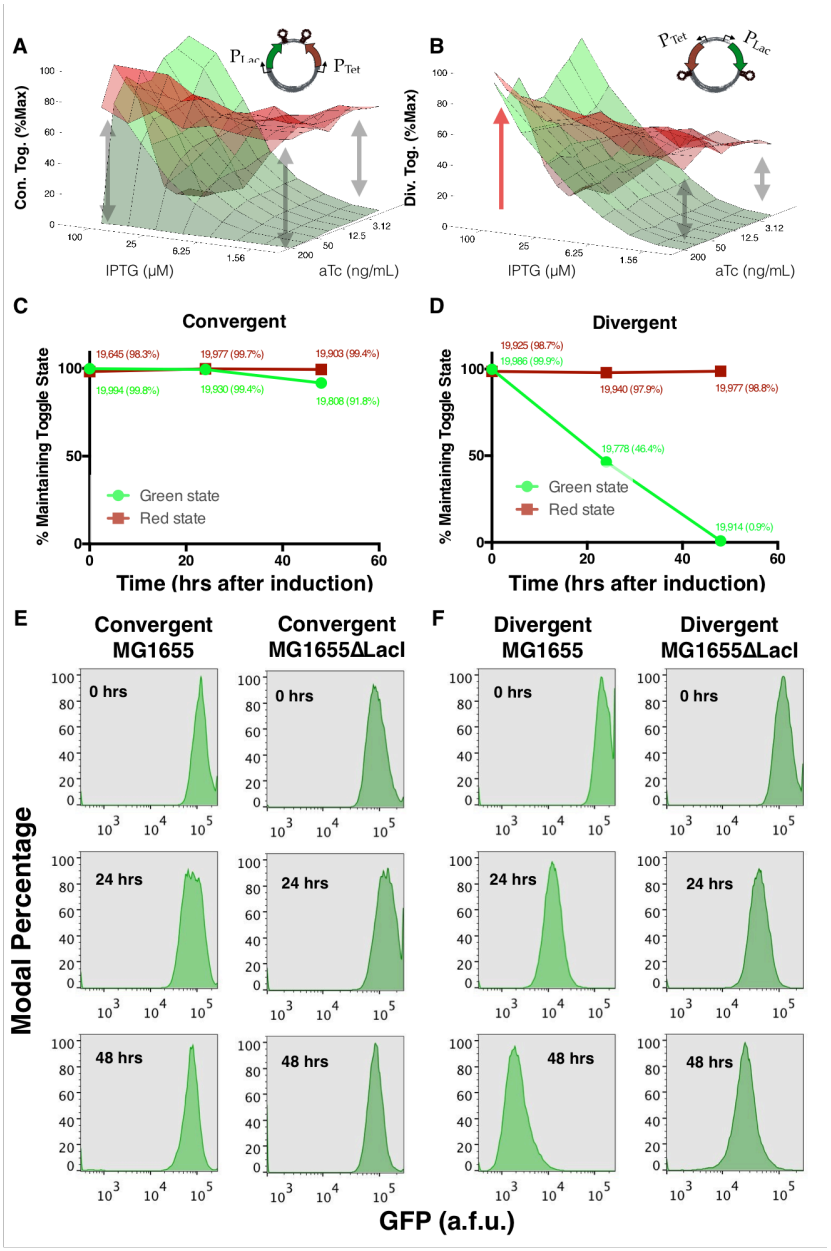

Figure S2: Related to Figure 7. (A) Experimental data from a dual reporter expression assay, titrating both IPTG and aTc concentrations to evaluate threshold behavior of the convergent Gardner-Collins toggle switch in MG1655LacI *E. coli*. **(B)** Experimental data from a dual reporter expression assay, titrating both IPTG and aTc concentrations to evaluate threshold behavior of the divergent Gardner-Collins toggle switch in MG1655LacI *E. coli*. **(C-D)** A stability test of the original Gardner- Collins toggle switch and its convergent counterpart in MG1655 *E. coli*. Cells were latched for 24 hours prior to the start of the experiment (t = 24 to t = 0) and subsequently rediluted in inducer-free media to assess stability of the toggle. The fraction of cells maintaining the original on-state are plotted against time. **(E)** Distributions showing stability of convergent toggle in the high GFP state in cell populations of MG1655 *E. coli* and MG1655LacI *E. coli* plotted at t = 0, 24, and 48 hours. **(F)** Distributions showing stability of divergent toggle in the high GFP state in cell populations of MG1655 *E. coli* and MG1655LacI *E. coli* plotted at t = 0, 24, and 48 hours.