Supplementary Information
(Anderson *et al.*, *Molecular Systems Biology*, 2007)

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I. Derivation of the AND-gate transfer function

Signal integration occurs in the AND gate via translation control. Several models of mRNA translation have been proposed and our analysis is based on this foundation (Gilchrist, 2006). The total rate r_x of production of activator protein can be expressed as a function of the rate of protein synthesis from a single mRNA k_x and the concentration of mRNA m,

$$r_x = k_x m \tag{1}$$

The single mRNA protein production rate is proportional to the probability σ that a ribosome will successfully synthesize a complete polypeptide. This can be discretized as the product of the probabilities σ_i that each amino acid i will be progressively added to a growing peptide of length N.

$$\sigma = \prod_i^N \sigma_i \tag{2}$$

In response to a TAG codon, either release factor 1 (the concentration of which is held constant) or SupD can enter the A site of the ribosome resulting in termination or nonsense suppression. The nonsense suppression rate r_i is given by

$$r_i = k_s s \tag{3}$$

where s is the concentration of SupD and k_s is a rate constant. The probability of suppression is therefore

$$\sigma_i = \frac{k_s s}{r_0 + k_s s} \tag{4}$$

where r_0 is the rate of termination. All other codons are assumed to not result in premature termination of translation (σ_i=1) (Gilchrist et al, 2006). Because there are two amber stop codons in the open reading frame, the probability that a polypeptide will be synthesized is given by

$$\sigma = \left(\frac{k_s s}{r_0 + k_s s} \right)^2 \tag{5}$$

Equations (1), (2), and (4) yield the relationship between the total protein production rate and the concentration of SupD,

$$r_x = k_x m\left(\frac{k_s s}{r_0 + k_s s}\right)^2 \tag{6}$$

where k_P is the maximum rate of protein synthesis under complete suppression.

The production of activator *x* can be modeled with a differential equation tracking the production and degradation rates

$$\frac{dx}{dt} = r_x - \gamma_x x \tag{7}$$

where γ_x is the degradation rate of the activator. The production of an output gene *y* from the promoter acted on by the activator (the T7 promoter) is captured by

$$\frac{dy}{dt} = k_y \frac{x}{K + x} - \gamma_y y \tag{8}$$

where k_y and γ_y are production and degradation rates and K is the dissociation constant for activator binding to the promoter. Solving for the steady-state solution of (7) and (8) and inserting the expression (6) yields

$$\frac{y}{y_{\max}} = \frac{\frac{k_x}{\gamma_x}\left(\frac{k_s s}{r_0 + k_s s}\right)^2 m}{K + \frac{k_x}{\gamma_x}\left(\frac{k_s s}{r_0 + k_s s}\right)^2 m} \tag{9}$$

where $y_{\max} = k_y/\gamma_y$. The variables can be rescaled by $\alpha = sk_s r_0^{-1}$, $\beta = mK^{-1}$, and $\theta = \gamma_x k_x^{-1}$ to produce the dimensionless form,

$$\frac{y}{y_{\max}} = \frac{\alpha^2\beta}{\theta(1+\alpha)^2 + \alpha^2\beta} \tag{10}$$

Equation 10 describes the transfer function of the AND gate with two inputs.

To parameterize this model, both inputs are varied and the output fluorescence is measured. The activity of the input promoters are varied by changing the concentration of the arabinose and salicylate inducers. However, these concentrations are specific to these two inducible systems. Instead of using arabinose and salicylate concentrations as the inputs, the P_{BAD} and P_{sal} promoters are transcriptionally fused to green fluorescent protein and the fluorescence is measured as the inducer concentration is varied. The fluorescence units from these one-dimensional experiments are combined with the two-dimensional data to parameterize the transfer function. The advantage of this approach is that the fit parameters can be used to predict different promoters can be connected to the AND gate.

To fit to the fluorescence data, it is assumed that there is a linear relationship between the arbitrary units of fluorescence and *y*, *a*, and *b*. Putting $\alpha = z_1 I_1$, $\beta = z_2 I_2$, and $y = z_3 g$ into Eq. (10) produces

$$\frac{G}{G_{\max}} = \frac{I_1^{\,2} I_2}{a(b + I_1)^2 + I_1^{\,2} I_2} \qquad (11)$$

where $a = \theta z_1^{-1} z_2^{-1}$ and $b = z_1^{-1}$. The one-dimensional (I_1 and I_2) and two-dimensional (G/G_{max}) data is used to determine the parameters a and b. The linear assumption is not strong as any non-linear affects will be consistent for different promoter inputs.

Table S1: Parameters used in transfer function model

Name	Description	Units[1]
a	empirical fit parameter for 2-AND gate transfer function = $\theta z_1^{-1} z_2^{-1}$	au^2
α	dimensionless *supD* tRNA concentration = $s k_s r_0^{-1}$	-
b	empirical fit parameter for 2-AND gate transfer function = z_1^{-1}	au
β	dimensionless activator mRNA	-
γ_x	degradation rate of activator protein	T^{-1}
γ_y	degradation rate of output gene product	T^{-1}
G	fluorescence measured for gfp output of AND gate	au
G_{max}	maximum fluorescence	au
I_1	fluorescence measured from input promoter 1 in a standard plasmid	au
I_2	fluorescence measured from input promoter 2 in a standard plasmid	au
K	dissociation equilibrium constant for activator binding to output promoter	C
k_s	rate constant for SupD suppression of amber stop codon	$C^{-1}T^{-1}$
k_x	rate constant for activator expression	T^{-1}
k_y	rate constant for ouput gene product production	CT^{-1}
m	concentration of activator mRNA	C
N	number of codons in activator gene	-
θ	dimensionless parameter = $\gamma_x k_x^{-1}$	-
r_0	rate of termination due to an amber stop codon	T^{-1}
r_i	nonsense suppression rate	T^{-1}
r_x	rate of production of activator protein	CT^{-1}
s	concentration of supD tRNA	C
σ	probability that a full activator protein is produced after translation initiation	-
σ_i	probability that the ribosome will translate codon i	-
t	time	T
x	concentration of activator protein	C
y	concentration of gene product (output of AND gate)	C
y_{max}	maximum concentration of gene product	C
z_1	scaling factor to convert from I_1 fluorescent units to α	au^{-1}
z_2	scaling factor to convert from I_2 fluorescent units to β	au^{-1}
z_3	scaling factor to convert from y and y_{max} fluorescent units to g and g_{max}	au^{-1}

1. au: arbitrary units of fluorescence, C: concentration, T: time, -: no dimensions.

II. Fit of experimental data to the transfer function model

The experimental data for the AND gate was fit to the theoretical transfer function model (Equation 11) to obtain the parameters *a* and *b*. The normalized two-dimensional fluorimetry data is used as the circuit output (G/G_{max}). The inputs are obtained from the one-dimensional fluorimetry data for different concentrations of inducer (Input 1: P_{sal}, Input 2: P_{BAD}). This data was fit to the model using the non-linear regression algorithm provided in the XLSTAT statistics software package. Only the data for the circuit variant exhibiting AND gate behavior (B9) was used to fit the parameter (Figure S1). The Pearson correlation coefficient for this fit is 0.971. The fit to the variant that did not exhibit AND-gate behavior (F11) is also shown (Figure S2). The Pearson correlation coefficient for this fit is 0.642.

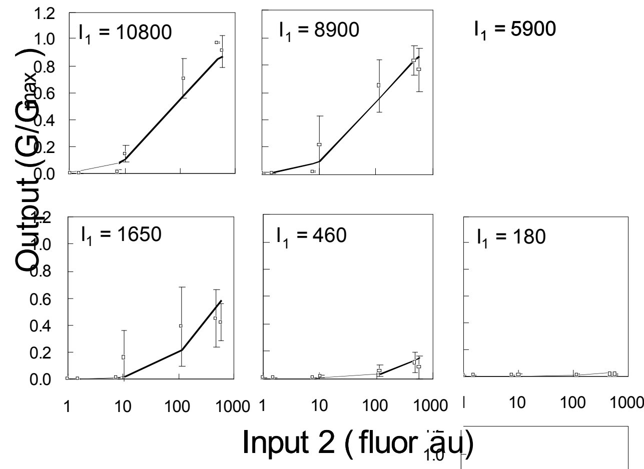

Figure S1: The circuit exhibiting AND gate behavior (B9) is fit to the transfer function model. Each graph shows the induction of the circuit with respect to Input 2 (P_{BAD}) at constant Input 1 (P_{sal}). Both of the inputs are shown in arbitrary units of fluorescence from the one-dimensional fluorimetry data. The output G/G_{max} is the normalized fluorescence from the two-dimensional fluorimetry data. The error is shown as the standard deviation from four experiments on different days.

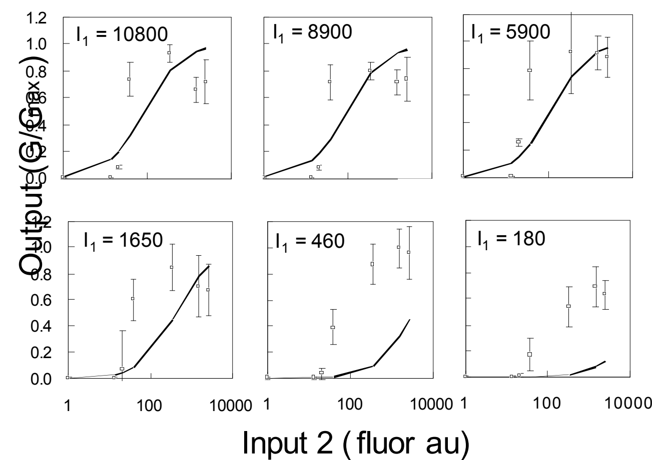

Figure S2: Data for the circuit not exhibiting AND gate behavior (F11) is shown. The model was parameterized using the B9 data (Figure S1).

III. Circuit influence on cell growth

The impact of the circuit on cell growth was determined. Experiments were performed both when the circuit is in the OFF (neither inducer is present) and the ON (both inducers are present) states. In the ON state, the circuit is expressing both the T7 RNA polymerase and the SupD suppressor tRNA. The maximum expression of these components does not significantly affect the growth rate (Figure S3) or the morphology of the cells (Figure S4).

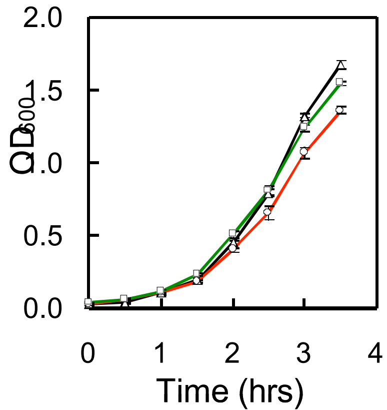

Figure S3: The effect of the AND gate on cell growth is minimal. The growth curves are shown for wild-type MC1061 (top, black), and cells containing the circuit in the OFF state (middle, green), and the circuit in the ON state (bottom, red). The circuit is turned on by the addition of the maximum amount of arabinose and salicylate. Data is shown for four replicates. Overnight starter cultures of MC1061 and pAC-SalSer914/pBACr-AraT7940 B9/MC1061 were used to inoculate flasks of 100mL 2YT media supplemented with nothing, Kan/Cm (25ug/mL each), or Kan/Cm and Sal/Ara (100ug/mL each). 2mL of MC1061 culture was added to the first flask. The other two flasks received 2mL of pAC-SalSer914/pBACr-AraT7940. The flasks were grown at 37°C with shaking. The error bars represent the standard deviation from five growth experiments.

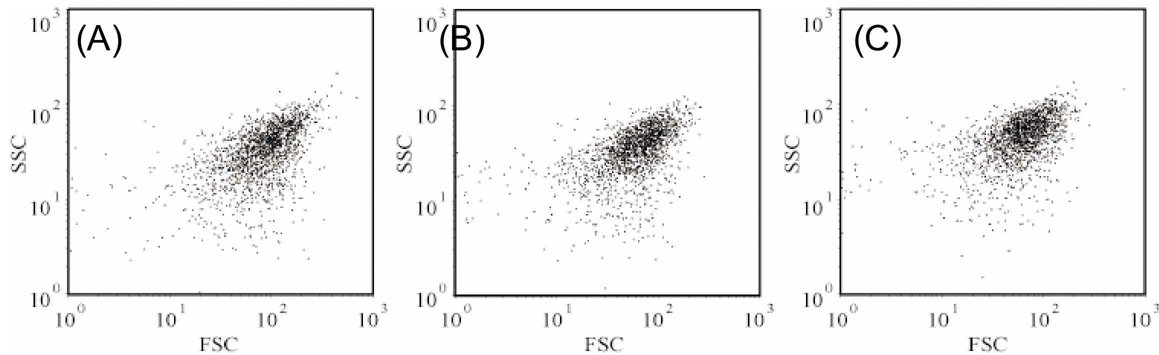

Figure S4: The AND gate does not significantly alter the forward and side scatter of the cytometry data. Cytometry data is shown for: (A) MC1061 alone, (B) cells harboring the AND gate in the off state, and (C) cells harboring the AND gate in the ON state. The experiments were performed as detailed in Figure S1. At OD= 1.0, aliquots of each culture were subjected to cytometry on a Partec instrument. For each data point, 30000 counts were collected, but only 2000 are shown.

IV. Response of the P_{BAD} promoter

The P_{BAD} promoter exhibits both all-or-none and graded behavior in *E. coli* MC1061 on the BAC backbone. The all-or-none response only occurs at the transition point. Before and after the transition, the cells are induced in a graded manner. This is consistent with previous experiments of P_{BAD} in MC1061. (Siegele et al, 1997) The population heterogeneity at the transition point does not affect the fit to the transfer function model (Figure S1).

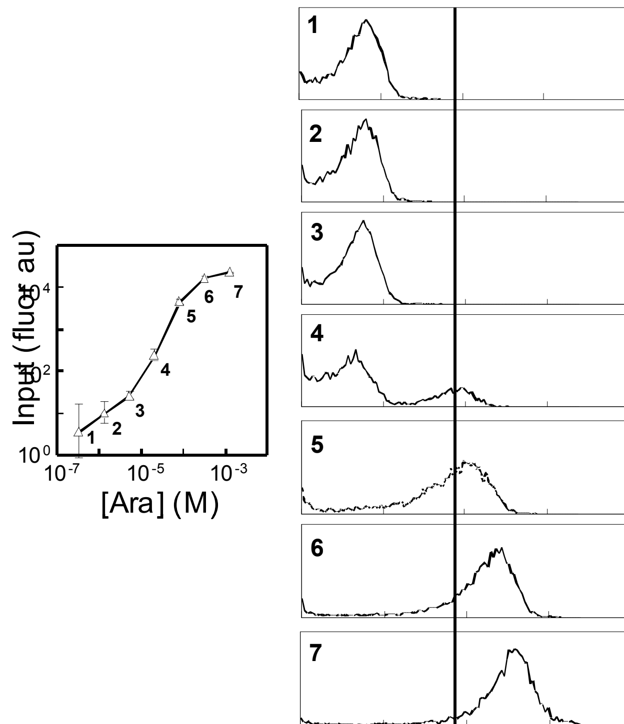

Figure S6: The population behavior is shown for the P_{BAD} promoter in the BAC backbone in *E. coli* MC1061. The fluorimentry data is shown (corresponding to Figure 3B) on the left and the cytometry data for each point is shown at the right. Each distribution contains 3000 gated cells.

VI. Screening Data

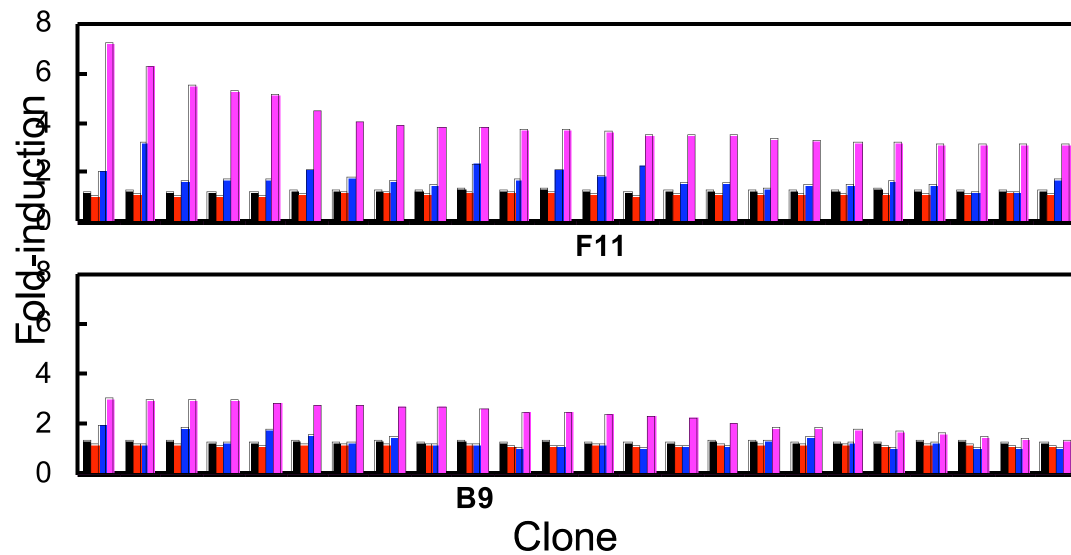

Figure S7: Screening data is shown for 48 ribosome binding site clones. Each clone was grown in four conditions and measured in the fluorimeter (black bar: no inducer, red bar: salicylate only, blue bar: arabinose only, purple bar: both salicylate and arabinose). The F11 and B9 clones were chosen for further analysis.

V. Plasmid and Construct Maps

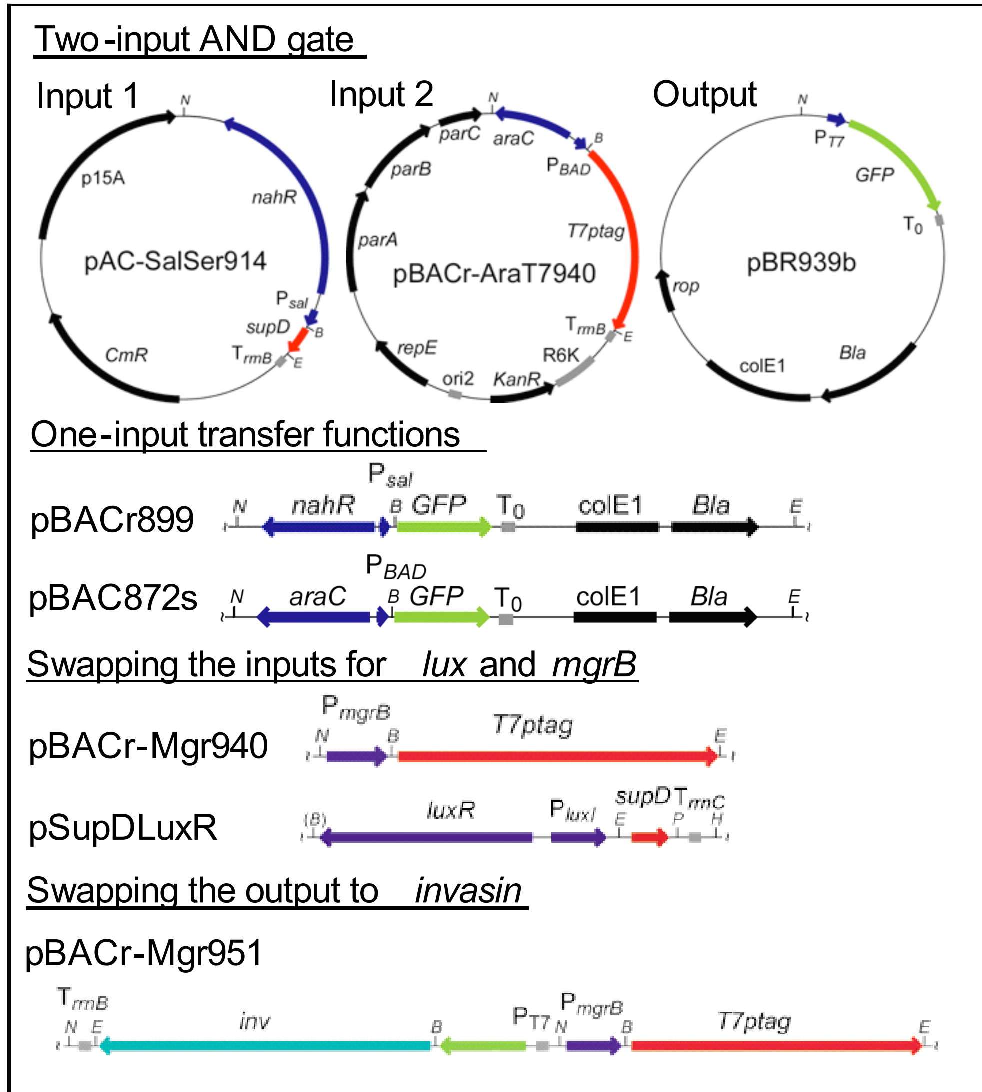

Figure S8: The plasmids and constructs used in this study are shown. Each input and the output are maintained on separate plasmids (top). The plasmids used for the one-dimensional transfer functions are were constructed based on pBACr. Three ribosome binding site variants of the PBAD promoter were constructed (pBAC872s contains the wt rbs, pBAC987 contains the B9 rbs, and pBAC978 contains the F11 rbs). The inputs were swapped by modifying pAC-SalSer914 to pSupDLuxR and pBAC-AraT7940 to pBACr-Mgr940. The output was swapped by modifying pBACr-AraT7940 to contain both the mgrB input promoter and the invasion output. The Restriction sites for *Not*I, *Bam*HI, *PstI*, and *Eco*RI are indicated as N, B, P, and E respectively.

Table S2: Plasmids used in this study

Plasmid	Origin	Resistance	Features
pAC581	p15A	CmR	P_{tet} Promoter
pAC-SalSer914	p15A	CmR	P_{sal} Promoter, *supD*
pSupDLuxR	p15A	CmR	*luxR,* P_{luxI} Promoter, *supD*
pBAC874t	BAC, R6K, colE1	KnR, Amp	P_{tet} Promoter, *GFPmut3*
pBAC872s	BAC, R6K, colE1	KnR, Amp	P_{BAD} promoter, *GFPmut3*
pBAC978	BAC, R6K, colE1	KnR, Amp	P_{BAD} promoter, *GFPmut3*
pBAC987	BAC, R6K, colE1	KnR, Amp	P_{BAD} promoter, *GFPmut3*
pBACr-Mgr901	BAC, R6K, colE1	KnR, Amp	P_{mgrB} Promoter, *GFPmut3*
pBACr-AraT7940	BAC,R6K	KnR	P_{BAD} promoter, *T7ptag*
pBACr-Mgr940	BAC,R6K	KnR	P_{mgrB} Promoter, *T7ptag*
pBACr-Mgr951	BAC,R6K	KnR	P_{mgrB} Promoter, *T7ptag*, P_{T7} Promoter, *GFPmut3_LAA*, *inv*
pBACr-AraGFP	BAC,R6K	KnR	P_{BAD} Promoter, *GFPmut3*
pBR939B	colE1	Amp	P_{T7} Promoter, *GFPmut3_LAA*

Table S3: Oligonucleotides used in this study

Name	Sequence
ca279	GCATTACGCTGACTTGACGGG
ca564R	CTAGCGGATCCTTCCTGCTCGCCTAACAGC
ca606R	GTCGACGGCGCTATTCAGATCCTC
ca721R	CACTGGAATTCGTAATGACAGATAATTTTACTC
ca742F	CAGTCGGATCCTTAATTTTTAAAGTATGGGCAATC
ca747R	CACTGAGATCTGCGTTTATTCGACTATAACAAAC
ca752F	GGTCAAGATCTGTATTGTCTATGCCTATTAATG
ca899F	GTGAAGCGGCCGCTGCGATCCCGCGAAGAACC
ca899R	GCAAAGGATCCTCTATGGTACTCGTGATGGC
ca901F	GTGAAGCGGCCGCGATGAGAGTAAGAACCTGTC
ca901R	GCAAAGGATCCTGCGCCCAAAGCAGCAAGC
ca914F	CACTAGGATCCAATTCGGAGAGATGCCGGAG
ca914R	GCTCTGAATTCAGCTTAAAAAAAAATCCTTAG
ca940F	CAGGTGGATCCNNNGGAATTAACCRTGACCATGATTACCGTGCAC
ca951R	GTGAAGCGGCCGCGTTCACCGACAAACAACAG
ca978F	CTTGAGGATCCCTAGGAATTAACCGTGAGTAAAGGAGAAGAACTTTTC
ca987F	CTTGAGGATCCAAAGGAATTAACCGTGAGTAAAGGAGAAGAACTTTTC