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 $r_s$ 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 $m$,

$$r_s = k_x m$$  \hspace{1cm} (1)

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

$$\sigma = \prod_{i=1}^{N} \sigma_i$$  \hspace{1cm} (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

$$r_i = k_s s$$  \hspace{1cm} (3)

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

$$\sigma_i = \frac{k_s s}{r_0 + k_s s}$$  \hspace{1cm} (4)

where $r_0$ is the rate of termination. All other codons are assumed to not result in premature termination of translation ($\sigma_i=1$) (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

$$\sigma = \left( \frac{k_s s}{r_0 + k_s s} \right)^2$$  \hspace{1cm} (5)

Equations (1), (2), and (4) yield the relationship between the total protein production rate and the concentration of SupD,
\[ r_x = k_x m \left( \frac{k_x s}{r_0 + k_x s} \right)^2 \]  

(6)

where \( k_x \) 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

\[ \frac{dx}{dt} = r_x - \gamma_x x \]  

(7)

where \( \gamma_x \) 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

\[ \frac{dy}{dt} = k_y \frac{x}{K + x} - \gamma_y y \]  

(8)

where \( k_y \) and \( \gamma_y \) 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

\[ y = \frac{k_x \left( \frac{k_x s}{r_0 + k_x s} \right)^2 m}{\frac{k_x \left( \frac{k_x s}{r_0 + k_x s} \right)^2 m}{K + \frac{k_x \left( \frac{k_x s}{r_0 + k_x s} \right)^2 m}} \]  

(9)

where \( y_{max} = k_y / \gamma_y \). The variables can be rescaled by \( \alpha = sk_r^{-1} \), \( \beta = mK^{-1} \), and \( \theta = \gamma_k^{-1} \) to produce the dimensionless form,

\[ \frac{y}{y_{max}} = \frac{\alpha^2 \beta}{\theta(1 + \alpha^2) + \alpha^2 \beta} \]  

(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 \( \alpha = z_1 l_1 \), \( \beta = z_2 l_2 \), and \( y = z_3 g \) into Eq. (10) produces
\[ \frac{G}{G_{\text{max}}} = \frac{I_1^2 I_2}{a(b + I_1)^2 + I_1^2 I_2} \]  

(11)

where \( a = \theta z_1^{-1} z_2^{-1} \) and \( b = z_1^{-1} \). The one-dimensional \((I_1, I_2)\) and two-dimensional \((G/G_{\text{max}})\) 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
|------|------------------------------------------------------------------------------|------|
| \( a \) | empirical fit parameter for 2-AND gate transfer function = \( \theta z_1^{-1} z_2^{-1} \) | \( \text{au} \^{-1} \)
| \( \alpha \) | dimensionless \( supD \) tRNA concentration = \( sk \sigma_0^{-1} \) | - |
| \( b \) | empirical fit parameter for 2-AND gate transfer function = \( z_1^{-1} \) | \( \text{au} \)
| \( \beta \) | dimensionless activator mRNA | - |
| \( \gamma_x \) | degradation rate of activator protein | \( \text{T}^{-1} \)
| \( \gamma_y \) | degradation rate of output gene product | \( \text{T}^{-1} \)
| \( G \) | fluorescence measured for gfp output of AND gate | \( \text{au} \)
| \( G_{\text{max}} \) | maximum fluorescence | \( \text{au} \)
| \( I_1 \) | fluorescence measured from input promoter 1 in a standard plasmid | \( \text{au} \)
| \( I_2 \) | fluorescence measured from input promoter 2 in a standard plasmid | \( \text{au} \)
| \( K \) | dissociation equilibrium constant for activator binding to output promoter | \( \text{C} \)
| \( k_x \) | rate constant for \( supD \) suppression of amber stop codon | \( \text{C}^{-1} \text{T}^{-1} \)
| \( k_x \) | rate constant for activator expression | \( \text{T}^{-1} \)
| \( k_y \) | rate constant for output gene product production | \( \text{CT}^{-1} \)
| \( m \) | concentration of activator mRNA | \( \text{C} \)
| \( N \) | number of codons in activator gene | - |
| \( \theta \) | dimensionless parameter = \( \gamma_x k_x^{-1} \) | - |
| \( r_0 \) | rate of termination due to an amber stop codon | \( \text{T}^{-1} \)
| \( r_i \) | nonsense suppression rate | \( \text{T}^{-1} \)
| \( r_x \) | rate of production of activator protein | \( \text{CT}^{-1} \)
| \( s \) | concentration of \( supD \) tRNA | \( \text{C} \)
| \( \sigma \) | probability that a full activator protein is produced after translation initiation | - |
| \( \sigma_i \) | probability that the ribosome will translate codon \( i \) | - |
| \( t \) | time | \( \text{T} \)
| \( x \) | concentration of activator protein | \( \text{C} \)
| \( y \) | concentration of gene product (output of AND gate) | \( \text{C} \)
| \( y_{\text{max}} \) | maximum concentration of gene product | \( \text{C} \)
| \( z_1 \) | scaling factor to convert from \( I_1 \) fluorescent units to \( \alpha \) | \( \text{au}^{-1} \)
| \( z_2 \) | scaling factor to convert from \( I_2 \) fluorescent units to \( \beta \) | \( \text{au}^{-1} \)
| \( z_3 \) | scaling factor to convert from \( y \) and \( y_{\text{max}} \) fluorescent units to \( g \) and \( g_{\text{max}} \) | \( \text{au}^{-1} \)

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 ($G/G_{\text{max}}$). The inputs are obtained from the one-dimensional fluorimetry data for different concentrations of inducer (Input 1: $P_{\text{sal}}$, Input 2: $P_{B4D}$). 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 ($P_{B4D}$) at constant Input 1 ($P_{\text{sal}}$). Both of the inputs are shown in arbitrary units of fluorescence from the one-dimensional fluorimetry data. The output $G/G_{\text{max}}$ 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).

![Growth curves](image)

**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.

![Cytometry plots](image)

**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).

![Graph](image)

**Figure S6:** The population behavior is shown for the $P_{BAD}$ promoter in the BAC backbone in *E. coli* MC1061. The fluorimetry 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 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 *NstI*, *BamHI*, *PstI*, and *EcoRI* are indicated as N, B, P, and E respectively.
### Table S2: Plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Origin</th>
<th>Resistance</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAC581</td>
<td>p15A</td>
<td>CmR</td>
<td>( P_{tet} ) Promoter</td>
</tr>
<tr>
<td>pAC-SalSer914</td>
<td>p15A</td>
<td>CmR</td>
<td>( P_{sal} ) Promoter, ( supD )</td>
</tr>
<tr>
<td>pSupDLuxR</td>
<td>p15A</td>
<td>CmR</td>
<td>( luxR ), ( P_{luxI} ) Promoter, ( supD )</td>
</tr>
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<td>pBAC874t</td>
<td>BAC, R6K, colE1</td>
<td>KnR, Amp</td>
<td>( P_{tet} ) Promoter, ( GFPmut3 )</td>
</tr>
<tr>
<td>pBAC872s</td>
<td>BAC, R6K, colE1</td>
<td>KnR, Amp</td>
<td>( P_{BAD} ) Promoter, ( GFPmut3 )</td>
</tr>
<tr>
<td>pBAC978</td>
<td>BAC, R6K, colE1</td>
<td>KnR, Amp</td>
<td>( P_{BAD} ) Promoter, ( GFPmut3 )</td>
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<td>pBAC987</td>
<td>BAC, R6K, colE1</td>
<td>KnR, Amp</td>
<td>( P_{BAD} ) Promoter, ( GFPmut3 )</td>
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<tr>
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<td>BAC, R6K, colE1</td>
<td>KnR, Amp</td>
<td>( P_{mgrB} ) Promoter, ( GFPmut3 )</td>
</tr>
<tr>
<td>pBACr-AraT7940</td>
<td>BAC, R6K</td>
<td>KnR</td>
<td>( P_{BAD} ) Promoter, ( T7ptag )</td>
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<td>pBACr-Mgr940</td>
<td>BAC, R6K</td>
<td>KnR</td>
<td>( P_{mgrB} ) Promoter, ( T7ptag )</td>
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<td>BAC, R6K</td>
<td>KnR</td>
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<tr>
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<tr>
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<td>colE1</td>
<td>Amp</td>
<td>( P_{T7} ) Promoter, ( GFPmut3_LAA )</td>
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### Table S3: Oligonucleotides used in this study

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
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</tr>
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<tbody>
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<td>ca279</td>
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<td>ca564R</td>
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<tr>
<td>ca747R</td>
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<td>ca901F</td>
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