# Appendices

1. Derivation of the output function for the AND Gate module[1]: (modified from original work by Anderson et al.(2009))

The total rate $r_x$ of production of functional T7 polymerase without the effects of amber mutation can be viewed as a first-order reaction which is expressed as a product 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 $\sigma$ that a ribosome will bypass the effects of amber mutation and successfully synthesize a complete polypeptide. For a single-strand mRNA with a length of N nucleotides, $\sigma$ can be obtained by multiplying the probabilities( $\sigma_i$) for each amino acid residueto be successfully added to the growing peptide(assuming that each addition is independent of the previous one):

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

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. Assuming that the suppressing process is first-order(the concentration of the target mRNA remains constant), the nonsense suppression rate $r_i$ is given by

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

wheres is the concentration of the SupDtRNA(proportional to the strength of the $P_{sal}$ promoter, i.e. the strength of input 1) 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 ( $\sigma_i$ =1) (Gilchrist et al, 2006). Because there are two amber stop codons in the open reading frame, we have:

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

Combining equations (1), (2), and (4), the relationship between the total protein production rate and the concentration of SupD may be obtained:

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

where$k_P$ is the maximum rate of functional T7ptag protein synthesis under complete suppression.

The production of theT7ptag can be modeled with a differential equation which takes into the consideration the protein synthesis and degradation rates(degradation is viewed as a first-order reaction)

$$\frac{dP}{dt} = r_P - \gamma_P P \tag{7}$$

where $\gamma_P$ 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

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

where $k_y$ and $\gamma_y$ are production and degradation rates and K is the dissociation constant for activator binding to the promoter. The steady-state solution of (7) and (8) and inserting the expression (6) gives

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

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

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

Since the output is measured in units of fluorescence, two scaling factors, $z_1$ and $z_2$, are used to convert the absolute values of the output in (10) to au(arbitrary unit of fluorescence):

$\alpha = z_1 I_1$, $\beta = z_2 I_2$, and $y = z_3 G$

By replacing the above terms in equation (10), we have:

$\frac{G}{G_{\max}} = \frac{I_1^2 I_2}{a(b+I_1)^2 + I_1^2 I_2}$ , in which $a = \theta z_1^{-1} z_2^{-1}$ and $b = z_1^{-1}$are parameters to be fitted using experimental values of $I_1$, $I_2$ and G($G_{max}$).

2. Relationship between *a* and △G:

Because $a = \theta z_1^{-1} z_2^{-1} = \gamma_P k_P^{-1} z_1^{-1} z_2^{-1}$, in which $k_P = K\exp\{-\beta\Delta G\}$(See the modeling for the RBS Calculator), $a \propto 1/K\exp\{-\beta\Delta G\}$. Therefore, all values of *a* may be derived after one absolute value for a sequence(whose ΔG is determined by the RBS Calculator) has been obtained(fitted value of 50, according to Anderson et al.).

3. Testing the robustness of our AND gate modeling

In order to quantitatively predict and modulate the behavior of the AND gate,we employed the terms $F_{AS}/F_0$, $F_{AS}/F_A$, and $F_{AS}/F_S$, which correspond to (1) the ratio of the output when both inputs are present to that when both inputs are absent; (2)the ratio of the output when both inputs are present to that when only input 1(arabinose) is present; (3)the ratio of the output when both inputs are present to that when only input 2(salicylate) is present. A good AND gate should display high (and nearly equal) values for all three terms.

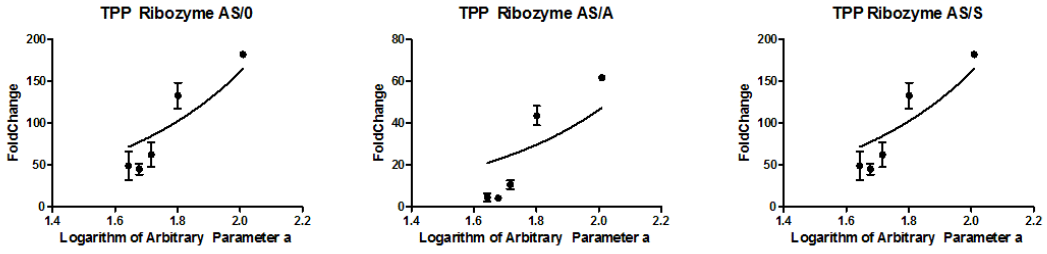
By replacing the F values in above three terms in equation (2) and simplifying the final expression, we have:

$$\frac{F_{AS}}{F_0}=\frac{\frac{a}{K_{AS}}+1}{\frac{a}{K_0}+1},\quad \frac{F_{AS}}{F_A}=\frac{\frac{a}{K_{AS}}+1}{\frac{a}{K_A}+1} \text{ and } \frac{F_{AS}}{F_S}=\frac{\frac{a}{K_{AS}}+1}{\frac{a}{K_S}+1},$$

where $K_{AS}=\frac{I_{1\max}I_{2\max}^2}{(b+I_{1\max})^2}$, $K_A=\frac{I_{1\max}I_{2\min}^2}{(b+I_{1\max})^2}$, $K_S=\frac{I_{1\min}I_{2\max}^2}{(b+I_{1\min})^2}$ and $K_0=\frac{I_{1\min}I_{2\min}^2}{(b+I_{1\min})^2}$.

In the above equations, the three ratios are all functions of the parameter *a* alone(therefore functions of the RBS strength). All *K* values are constant by definition and are therefore regarded as parameters in the above expressions.It is reasonably assumed that the two inputs are either at their maximal or minimal values as, in practice, either no inducer is added or a concentration of the inducer sufficient for fully inducing the promoter is applied.

Before predicting the behavior of the AND gate, we first validated the above functions by fitting them to experimental results. Using the ligand-responsive ribozyme as an RNA controller, we are able to quantitatively alter values of *a* via addition of varying concentrations of the ligand, according to the ligand response curve for the specific ribozyme(TPP ribozyme in our case), shown in Figure 10 in the main text. Since *a* possesses a quantitative relationship with $\Delta G_{tot}$ and may be calculated once its initial value(the state in which no extraneous regulation is placed upon translation initiation, i.e., the ribozyme is in a fully "ON" state) is determined using the RBS Calculator, we may convert the levels of reporter gene expression relative to the maximum absolute values of *a*. The experimental values of $F_{AS}/F_0$, $F_{AS}/F_A$, and $F_{AS}/F_S$ may also be calculated from the measured output fluorescence. Therefore, by plotting these values versus the theoretically determined values of *a* and fitting for $K_{AS}$, $K_A$,$K_S$ and $K_0$, it is possible to evaluate the fitness of our model to experimental results. The fitted curves are shown in Fig S1.




(a) (b) (c)

**Figure S1. Fitted curves for determination of $K_{AS}$, $K_A$, $K_S$ and $K_0$. It can be seen that all three ratios are high and approximately equal at low translation rates(high values of *a*), while low and vary significantly at high translation rates(low values of *a*).**

Reference

1. Anderson, J.C., Voigt, C.A., and Arkin, A.P.(2007). Environmental signal integration by a modular AND gate-Supplementary Information. Nature Molecular Systems Biology *3*,133.