Construction of two-input logic gates using transcriptional interference

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Mathematical Model Derivation

Transfer Functions for Promoter and Operator Occupancy

The equilibrium binding of a ligand to a transcription factor can be modeled through the Hill equation (Equation S1, same as Equation 4 in main text):

$$f_{aTc:T} = \frac{aTc^m}{K_d + aTc^m} \quad \text{(S1)}$$

Where *f* represents the fraction of transcription factor that is bound to the ligand, *m* is the Hill coefficient, *aTc* represents the ligand concentration—in this case, the concentration of aTc—and K_d is the dissociation constant of the ligand to the transcription factor (Fig. 4 and 7, Fig. S1). The fraction of free transcription factor is then determined using Equation S2 (same as Equation 5 in main text):

$$f_T = 1 - f_{aTc:T} \quad \text{(S2)}$$

The binding events, depicted in Fig. 4 for AND gate constructs and Fig. 7 for OR gate constructs, are represented in terms of the relevant protein concentrations and DNA dissociation constants. The model fitted values for the DNA dissociation constants are provided in Supplementary Table S22-S23 and in Supplementary Figures S11-S18, S25-S30 and literature-derived values are reported in Supplementary Table S23 in terms of the fraction of free protein that is not bound to a ligand. These states are described in Supplementary Figure S1, adapted from Tamsir *et al.*'s derivation (Tamsir *et al*, 2011) of transfer functions describing promoter states. GFP expression is a function of the inducer concentrations, which permits the observed logic gate behavior as a function of inducer concentrations.

We developed transfer function for pTet, pLac, and LacO utilizing the Shea-Ackers formalism (Shea & Ackers, 1985), a statistical mechanical approach used to describe DNA occupancy. The states describing the possible binding events for each part, depicted both in Figures 4a, 7a, and Supplementary Figure S1 for pTet, were arranged into transfer functions with states that permit transcription in the numerator and all possible states in the denominator.

In the case of LacO, shown in Equation S3a, the state that permits transcription is the lack of protein binding, denoted here with a 1, the binding repression term is shown in the denominator. The binding term is dependent on inducer through Equations S1 and S2, rendering this transfer function dependent on IPTG concentration. TF_{pLac} results from the inclusion of RNAP binding, where instead transcription occurs with the RNAP:pLac binding term, $K_{a,RNAP:pLac} * [RNAP]$, in the numerator (Equation S3b). TF_{pTet} contains a similar form to TF_{pLac}, but has additional terms capturing the binding dynamics of TetR to the two available TetO sites (Supplementary Figure S1).

$$\boldsymbol{TF_{LacO}} = \frac{1}{1 + (K_{a,LacI} * [LacI] * f_L)} \quad \text{(S3a)}$$

$$\boldsymbol{TF_{pLac}} = \frac{K_{a,RNAP:pLac} * [RNAP]}{1 + (K_{a,LacI} * [LacI] * f_L) + K_{a,RNAP:pLac} * [RNAP]} \quad \text{(S3b)}$$

$$\boldsymbol{TF_{pTet}} = \frac{K_{a,RNAP} * [RNAP]}{1 + (K_{a,TetR} * [TetR] * f_T)^2 + 2 * (K_{a,TetR} * [TetR] * f_T) + K_{a,RNAP} * [RNAP]} \quad \text{(S3c)}$$

Transfer functions describing promoter and operator sites are then combined into model equations that attempt to describe the behavior of a logic gate system. These model equations were fit to grids of log-transformed flow cytometry data of GFP expression measured at 5 different aTc and 5 different IPTG concentrations and the goodness of fit statistics were recorded. Model equations were quantitatively compared using the Akaike Selection Criterion (Bozdogan, 1987), as reported in Supplementary Tables S24-25.

AND Gate Model Equation Derivation

AND gates were first fitted using a model equation multiplying the two transfer functions, TF_{pTet}, TF_{LacO}, normalized through a parameter *k*, which accounts for translation. This equation is given as Equation S4:

$$GFP = k(TF_{pTet} * TF_{LacO}) \quad \text{(S4)}$$

However, this equation does not capture observed readthrough of RNAP through the LacI roadblock, which was observed in all AND constructs. Three models described by equations S5-S7, shown below, were developed in order to account for this readthrough.

$$GFP = k(a * TF_{pTet} * TF_{LacO} + (1 - a) * TF_{pTet} * (1 - TF_{LacO})) \quad (S5)$$

$$GFP = k(TF_{pTet} * TF_{LacO} + TF_{pTet}) \quad (S6)$$

$$GFP = k(a * TF_{pTet} * TF_{LacO} + (1 - a) * TF_{pTet}) \quad (S7)$$

Equation S5 effectively splits the GFP expression into true AND behavior for some fraction *a*, and RNAP from pTet reading through the LacI roadblock for some fraction (1-*a*), where *a* is a fitted parameter.

Equation S6 models AND gate behavior as a combination of a true AND gate with a single aTc gate, and Equation 4 splits these contributions into fractions represented by a fitted parameter, *a*.

For the majority of constructs, Equation S7 provided the best fit for the number of parameters, as indicated by AIC and reported in Supplementary Table S24. Hill coefficients were either fit or fixed at a value of 2—the name 'Hill' next to the equation number in Supplementary Table S24 indicates that the coefficients were fixed. Parameter values and 95% confidence intervals are reported in Supplementary Figures S11-S18.

OR Gate Model Equation Derivation

OR gates were fit using parameters from the AND gate with the corresponding operator site. For instance, in the case of pAE_LG25, the OR construct represented in the main text, all association constants except for $K_{LacI:pLac}$ Hill coefficients, and other coefficients unique to OR gate model equations, such as a_{pTet}, a_{pLac}, b, and c were obtained from the best fit of the AND gate containing the same operator site, pAE_LG06.

The tandem promoter OR constructs were initially modeled as a simple sum of the two promoter activities, shown in Equation S8:

$$GFP_{OR} = X_{pTet} * TF_{pTet} + X_{pLac} * TF_{pLac} \quad (S8)$$

Where X_{pTet} and X_{pLac} are the maximum promoter activities attributed to pTet and pLac, respectively. The downstream pLac activity, X_{pLac}, was obtained under the maximum IPTG and zero aTc condition; the upstream pTet activity, X_{pTet}, was obtained through mutations to the downstream pLac in both the -10, -35, and LacO regions that removed promoter activity and LacI binding activity.

Previously, Tamsir et al(Tamsir *et al*, 2011) proposed a model equation for OR gates that contains two terms describing the individual contributions of each tandem promoter with weighting terms a_{pTet} and a_{pLac} used to describe the interference of one promoter on another and the relative contributions of each promoter to GFP expression. This equation is represented in Equation S9.

$$GFP_{OR} = \boldsymbol{a}_{pTet} * X_{pTet} * TF_{pTet} + \boldsymbol{a}_{pLac} * X_{pLac} * TF_{pLac} \quad (S9)$$

These promoter weights suggest the relative importance of each promoter to GFP expression in the different OR constructs and are reported in Supplementary Table S23. As expected, the downstream pLac promoter is generally weighted more highly in all constructs, indicating that it contributes more to GFP expression in the presence of IPTG, which is in good agreement with experimental data shown in Figure 5b.

Through model selection using AIC, reported in Supplementary Table S25, we found that, for some constructs, such as pAE_LG25, this model equation performed best. But in some cases, the fits benefited from the addition of a third term, shown in the equation below:

$$GFP_{OR} = \boldsymbol{a}_{pTet} * X_{pTet} * TF_{pTet} + \boldsymbol{a}_{pLac} * X_{pLac} * TF_{pLac} + b * ([aTc] - c) * TF_{pTet} * TF_{pLac} \quad (S10)$$

Where *c* is some critical value at which the promoters cooperate and acquire an AND-like behavior. This OR behavior is represented by the multiplication of the two promoters' transfer functions, and the term itself is weighted by coefficient *b*. In all fits in which this model equation was determined the best, the value of *b* was relatively small, indicating that any possible cooperative effects between the two promoters are in some cases non-negligible.

Assignment of Logic Parameter Space

In order to assign the parameter space to each data point, we followed the Cox *et. al* model(Cox *et al*, 2007). First the GFP expression was ordered from highest to lowest by inducer condition (none, aTc only, IPTG only, aTc+IPTG) and labelled as follows: IV>III>II>I. Even though the GFP expression from two different conditions might not be significantly different, we have strictly followed this rule in order to thoroughly and completely assign proper logic parameter space for each construct under each inducer conditions. Otherwise, calculations of asymmetry, *a*, and logic, *l*, might yield values outside of their theoretical range.

Each logic parameter space was then defined as a triangular space with vertices corresponding to the logic parameters, *a*, the asymmetry of the gate inputs aTc and IPTG, and *l*, the logic behavior that each gate exhibits—i.e which inputs are on vs. off under different inducer conditions. These parameters are defined in Equations S11 and S12 and are further described in the Methods section. Both Equations S11 and S12 contain the dynamic range term, *r*, which is defined in Equation S13.

$$a = \frac{log_{10}(III) - log_{10}(II)}{log_{10}(r)} \quad \text{(S11)}$$

$$l = \frac{2 * log_{10}(IV) - log_{10}(II * III)}{2 * log_{10}(r)} \quad \text{(S12)}$$

$$r = \frac{IV}{I} \quad \text{(S13)}$$

The vertices of the triangle plots are located at a=0, l=0*;* a=0, l=1; and a=1, l=0.5, which correspond to extreme or ideal logic behaviors. If three conditions are ON and one is OFF (IV=III=II>I) then a=0, l=0; if one condition is ON and three are OFF IV>(III=II=I) then a=0, l=1; finally, if two conditions are ON and two are OFF (IV=III)>(II=I) then a=1, l=0.5.

The logic space assigned to each construct/inducer combination reflects its GFP expression profile—the ranked order of all inducer conditions. For example, if GFP expression profile is GFP_{a+I}>GFP_{aTc}>GFP_{IPTG}>GFP_{Basal}, then the logic parameter space will be OR-AND-aTc, representing the a=0, l=0*;* a=0, l=1; and a=1, l=0.5 vertices, respectively. For this GFP expression profile, the a=0, l=0 vertex (IV=III=II>I) corresponds to OR logic, where only the uninduced, basal condition outputs a 0 while the presence of any input outputs a 1. The a=0, l=1 vertex represents AND behavior because, given the GFP_{a+I}>GFP_{aTc}>GFP_{IPTG}>GFP_{Basal} expression profile, the IV>(III=II=I) state that defines the a=0, l=1 vertex represents ideal AND gate behavior characterized by only both inducers eliciting a 1 output. The state of the a=1, l=0.5, in this study, represents either a pure aTc or pure IPTG gate, since the (IV=III)>(II=I) condition indicates that the gate responds only to one inducer, aTc or IPTG.

Different GFP expression profiles will change the logic parameter space. For example, a GFP expression profile of GFP_{a+I}>GFP_{aTc}>GFP_{Basal}>GFP_{IPTG} is assigned to an aTc(IMPLY)IPTG-AND-aTc logic space, because the (IV=III=II>I) state here defines an aTc-IMPLY-IPTG gate. The truth tables for all observed logic spaces are reported in Supplementary Table S4. All observed logic parameter spaces based on the GFP expression profile can be found in Supplementary Table S5.GFP expression data with the corresponding *a*, *l*, *r* (regulatory range), logic parameter space

and logic behavior for each construct and combination of inducers can be found in Supplementary Tables S6-S21.

Supplementary Table S1: LacO sequences and their corresponding LacI K_D values. WT: Wild Type. Each O_1 sequence is underlined. Mutations are highlighted in red and bold. K_D values are taken from Betz *et al.*(Betz *et al*, 1986)

Construct	LacO Sequence	K_D (pM)
pAE_LG01 (WT)	TTGTGAGCGGATAACAAAAAGGCTTGTGAGCGGATAACAA	0.036
pAE_LG02	TTGTGAGCG**A**ATAACAAAAAGGCTTGTGAGCG**A**ATAACAA	0.018
pAE_LG03	TTGTGAGCG**C**ATAACAAAAAGGCTTGTGAGCG**C**ATAACAA	0.0092
pAE_LG04	TTGTGAG**GC**GATAACAAAAAGGCTTGTGAG**GC**GATAACAA	2.34
pAE_LG05	TTGTGA**C**CGGATAACAAAAAGGCTTGTGA**C**CGGATAACAA	0.15
pAE_LG06	TTGTGAGCGGA**C**AACAAAAAGGCTTGTGAGCGGA**C**AACAA	0.067
pAE_LG07	TT**A**TGAGCGGATAACAAAAAGGCTT**A**TGAGCGGATAACAA	0.6
pAE_LG13	TTGTGAGCGGA**G**AACAAAAAGGCTTGTGAGCGGA**G**AACAA	0.21

Supplementary Table S2: pLac sequences and their corresponding LacI K_D values. WT: Wild Type. Each O_1 sequence is underlined. Mutations are highlighted in red and bold. The transcriptional start site is double underlined. K_D values are taken from Betz *et al.*(Betz *et al*, 1986)

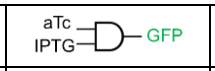
Constructs	pLac Sequence	K_D (pM)
pAE_LG15 pAE_LG21	TTGTGAGCGGATAACAATTGACATTGTGAGCGGATAACAAGATA CTGAGCACATCAGCAGGACGCACTGACT	0.036
pAE_LG23 pAE_LG25	TTGTGAGCGGA**C**AACAATTGACATTGTGAGCGGA**C**AACAAGATA CTGAGCACATCAGCAGGACGCACTGACT	0.067
pAE_LG26	TTGTGAGCGGA**G**AACAATTGACATTGTGAGCGGA**G**AACAAGAT ACTGAGCACATCAGCAGGACGCACTGACT	0.21
pAE_LG27	TTGTGAG**GC**GATAACAATTGACATTGTGAG**GC**GATAACAAGATA CTGAGCACATCAGCAGGACGCACTGACT	2.34

Supplementary Table S3: Sequence content of the fragments of different lengths separating pTet and pLac.

Constructs	pTet-pLac spacer	Length (bp)
pAE_LG15, pAE_LG23, pAE_LG26, pAE_LG27	ATCAGCAGGACGCACTGACCGAATTCATTAAAGAG GAGAAAGGTACC	47
pAE_LG21, pAE_LG25	ATCAGCAGGACGCACTGACCGAATTCGAATTGTGCG ATCCCTGCACCTCAGCTAAGGTAGCTACCAGGTACC	72

Characterization of Logic Parameter Space

Supplementary Table S4: Truth tables of logic gates defining the logic parameter spaces spanning between AND and OR logic.

						
aTc	IPTG	AND	(aTc) NIMPLY (IPTG)	(aTc) IMPLY (IPTG)	(IPTG) IMPLY (aTc)	OR
0	0	0	0	1	1	0
1	0	0	0	0	1	1
0	1	0	1	1	0	1
1	1	1	0	1	1	1

Supplementary Table S5: Definitions of logic parameter spaces based on GFP expression profile.

GFP expression profile				**Extreme behaviors**			
IV	**III**	**II**	**I**	***a*=0, *l*=0**	***a*=0, *l*=1**	***a*=1, *l*=0.5**	**Logic Space**
a+I	aTc	IPTG	Basal	OR	AND	aTc gate	OR-AND-aTc
a+I	aTc	Basal	IPTG	(aTc)IMPLY(IPTG)	AND	aTc gate	(a)I(I)-AND-aTc
aTc	a+I	IPTG	Basal	OR	(aTc)NIMPLY(IPTG)	aTc gate	OR-(a)N(I)-aTc
aTc	a+I	Basal	IPTG	(aTc)IMPLY(IPTG)	(aTc)NIMPLY(IPTG)	aTc gate	(a)I(I)-(a)N(I)-aTc
a+I	IPTG	aTc	Basal	OR	AND	IPTG gate	OR-AND-IPTG
IPTG	a+I	aTc	Basal	OR	(IPTG)NIMPLY(aTc)	IPTG gate	OR-(I)N(a)-IPTG
a+I	IPTG	Basal	aTc	(aTc)IMPLY(IPTG)	(IPTG)NIMPLY(aTc)	IPTG gate	(a)I(I)-(I)N(a)-IPTG

Supplementary Table S6: Mathematical characterization of logic gate behavior using the expanded Cox *et al.* model for all tested constructs designed to behave as AND gates (pTet-LacO architecture).

Construct	***l***	***a***	d_{10}	**Logic gate behavior**
pAE_LG01*	0.85	0.28	0.32	Asymmetric AND gate
pAE_LG02	0.73	0.52	0.59	Asymmetric AND gate
pAE_LG03*	0.77	0.41	0.47	Asymmetric AND gate
pAE_LG04	0.50	0.99	1.11	aTc gate
pAE_LG05	0.70	0.53	0.61	Asymmetric AND gate
pAE_LG06	0.66	0.66	0.75	Asymmetric AND gate
pAE_LG07	0.60	0.76	0.86	aTc gate
pAE_LG13	0.58	0.81	0.91	Asymmetric AND gate

* belong to the (a)I(I)-AND-aTc space.

Supplementary Table S7: Mathematical characterization of logic gate behavior using the expanded Cox *et al.* model for all tested constructs designed to behave as OR gates (pTet-pLac architecture).

Construct	***l***	***a***	d_{00}	**Logic gate behavior**
pAE_LG15	0.46	0.61	0.76	Asymmetric SLOPE gate
pAE_LG21	0.45	0.46	0.64	Asymmetric SLOPE gate
pAE_LG23	0.34	0.34	0.48	Asymmetric OR gate
pAE_LG25	0.36	0.32	0.49	Asymmetric OR gate
pAE_LG26	0.31	0.25	0.40	Asymmetric OR gate
pAE_LG27*	0.46	0.12	0.48	SLOPE gate

* belongs to the OR-AND-aTc space.

Supplementary Table S8: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG01. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	330	355	324	363	1.1	0.6	0.5	(a)I(I)-AND-aTc	Asym SLOPE gate
20	0.01	330	392	324	440	1.4	0.6	0.7	(a)I(I)-AND-aTc	Asym AND gate
30	0.01	330	484	324	631	1.9	0.6	0.7	(a)I(I)-AND-aTc	Asym AND gate
50	0.01	330	637	324	1216	3.8	0.5	0.7	(a)I(I)-AND-aTc	Asym AND gate
10	0.02	330	355	328	380	1.2	0.5	0.7	(a)I(I)-AND-aTc	Asym AND gate
20	0.02	330	392	328	484	1.5	0.4	0.8	(a)I(I)-AND-aTc	Asym AND gate
30	0.02	330	484	328	915	2.8	0.4	0.8	(a)I(I)-AND-aTc	Asym AND gate
50	0.02	330	637	328	2236	6.8	0.3	0.8	(a)I(I)-AND-aTc	Asym AND gate
10	0.5	330	355	332	438	1.3	0.2	0.9	OR-AND-aTc	AND gate
20	0.5	330	392	332	731	2.2	0.2	0.9	OR-AND-aTc	AND gate
30	0.5	330	484	332	1538	4.7	0.2	0.9	OR-AND-aTc	AND gate
50	0.5	330	637	332	3304	10.0	0.3	0.9	OR-AND-aTc	Asym AND gate
10	1	330	355	324	426	1.3	0.3	0.8	(a)I(I)-AND-aTc	Asym AND gate
20	1	330	392	324	872	2.7	0.2	0.9	(a)I(I)-AND-aTc	AND gate
30	1	330	484	324	1857	5.7	0.2	0.9	(a)I(I)-AND-aTc	AND gate
50	1	330	637	324	3299	10.2	0.3	0.9	(a)I(I)-AND-aTc	Asym AND gate

Supplementary Table S9: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG02. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	309	338	318	344	1.1	0.6	0.4	OR-AND-aTc	Asym SLOPE gate
20	0.01	309	407	318	459	1.5	0.6	0.6	OR-AND-aTc	Asym SLOPE gate
30	0.01	309	804	318	894	2.9	0.9	0.5	OR-AND-aTc	aTc gate
50	0.01	309	1434	318	2152	7.0	0.8	0.6	OR-AND-aTc	aTc gate
10	0.02	309	338	313	385	1.2	0.3	0.8	OR-AND-aTc	Asym AND gate
20	0.02	309	407	313	593	1.9	0.4	0.8	OR-AND-aTc	Asym AND gate
30	0.02	309	804	313	1269	4.1	0.7	0.7	OR-AND-aTc	Asym AND gate
50	0.02	309	1434	313	3129	10.1	0.7	0.7	OR-AND-aTc	Asym AND gate
10	0.5	309	338	317	404	1.3	0.2	0.8	OR-AND-aTc	Asym AND gate
20	0.5	309	407	317	1112	3.6	0.2	0.9	OR-AND-aTc	AND gate
30	0.5	309	804	317	1788	5.8	0.5	0.7	OR-AND-aTc	Asym AND gate
50	0.5	309	1434	317	5109	16.5	0.5	0.7	OR-AND-aTc	Asym AND gate
10	1	309	338	321	387	1.3	0.2	0.7	OR-AND-aTc	Asym AND gate
20	1	309	407	321	1369	4.4	0.2	0.9	OR-AND-aTc	AND gate
30	1	309	804	321	2171	7.0	0.5	0.7	OR-AND-aTc	Asym AND gate
50	1	309	1434	321	5543	17.9	0.5	0.7	OR-AND-aTc	Asym AND gate

Supplementary Table S10: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG03. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	399	433	471	535	1.3	0.3	0.6	OR-AND-IPTG	Asym SLOPE gate
20	0.01	399	656	471	792	2.0	0.5	0.5	OR-AND-aTc	Asym SLOPE gate
30	0.01	399	985	471	1613	4.0	0.5	0.6	OR-AND-aTc	Asym SLOPE gate
50	0.01	399	1339	471	3802	9.5	0.5	0.7	OR-AND-aTc	Asym AND gate
10	0.02	399	433	597	817	2.0	0.4	0.7	OR-AND-IPTG	Asym AND gate
20	0.02	399	656	597	1088	2.7	0.1	0.6	OR-AND-aTc	SLOPE gate
30	0.02	399	985	597	1820	4.6	0.3	0.6	OR-AND-aTc	Asym SLOPE gate
50	0.02	399	1339	597	5946	14.9	0.3	0.7	OR-AND-aTc	Asym AND gate
10	0.5	399	433	445	833	2.1	0.0	0.9	OR-AND-IPTG	AND gate
20	0.5	399	656	445	1897	4.8	0.2	0.8	OR-AND-aTc	Asym AND gate
30	0.5	399	985	445	3359	8.4	0.4	0.8	OR-AND-aTc	Asym AND gate
50	0.5	399	1339	445	8615	21.6	0.4	0.8	OR-AND-aTc	Asym AND gate
10	1	399	433	373	628	1.7	0.2	0.8	(a)I(I)-AND-aTc	AND gate
20	1	399	656	373	2034	5.5	0.3	0.8	(a)I(I)-AND-aTc	Asym AND gate
30	1	399	985	373	3413	9.2	0.4	0.8	(a)I(I)-AND-aTc	Asym AND gate
50	1	399	1339	373	7167	19.2	0.4	0.8	(a)I(I)-AND-aTc	Asym AND gate

Supplementary Table S11: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG04. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	313	387	313	372	1.2	0.8	0.6	(I)I(a)-(a)N(I)-aTc	aTc gate
20	0.01	313	952	313	965	3.1	1.0	0.5	(a)I(I)-AND-aTc	aTc gate
30	0.01	313	2118	313	1692	6.8	0.9	0.6	(I)I(a)-(a)N(I)-aTc	aTc gate
50	0.01	313	5802	313	4719	18.5	0.9	0.5	(I)I(a)-(a)N(I)-aTc	aTc gate
10	0.02	313	387	314	381	1.2	0.9	0.5	OR-(a)N(I)-aTc	aTc gate
20	0.02	313	952	314	720	3.0	0.7	0.6	OR-(a)N(I)-aTc	Asym SLOPE gate*
30	0.02	313	2118	314	2259	7.2	1.0	0.5	OR-AND-aTc	aTc gate
50	0.02	313	5802	314	4082	18.5	0.9	0.6	OR-(a)N(I)-aTc	aTc gate
10	0.5	313	387	319	389	1.2	0.9	0.5	OR-AND-aTc	aTc gate
20	0.5	313	952	319	933	3.0	1.0	0.5	OR-(a)N(I)-aTc	aTc gate
30	0.5	313	2118	319	1765	6.8	0.9	0.5	OR-(a)N(I)-aTc	aTc gate
50	0.5	313	5802	319	7184	22.9	0.9	0.5	OR-AND-aTc	aTc gate
10	1	313	387	328	384	1.2	0.7	0.4	OR-(a)N(I)-aTc	Asym SLOPE gate*
20	1	313	952	328	1074	3.4	0.9	0.5	OR-AND-aTc	aTc gate
30	1	313	2118	328	1867	6.8	0.9	0.5	OR-(a)N(I)-aTc	aTc gate
50	1	313	5802	328	5829	18.6	1.0	0.5	OR-AND-aTc	aTc gate

* Refers to the “asymmetric SLOPE (*a*=0.5, *l*=0.5) gate” in the OR-(a)N(I)-aTc space.

Supplementary Table S12: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG05. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	317	342	324	353	1.1	0.5	0.5	OR-AND-aTc	Asym SLOPE gate
20	0.01	317	408	324	425	1.3	0.8	0.5	OR-AND-aTc	aTc gate
30	0.01	317	481	324	507	1.6	0.8	0.5	OR-AND-aTc	aTc gate
50	0.01	317	745	324	957	3.0	0.8	0.6	OR-AND-aTc	aTc gate
10	0.02	317	342	327	356	1.1	0.4	0.5	OR-AND-aTc	Asym SLOPE gate
20	0.02	317	408	327	437	1.4	0.7	0.6	OR-AND-aTc	Asym SLOPE gate
30	0.02	317	481	327	535	1.7	0.7	0.6	OR-AND-aTc	Asym SLOPE gate
50	0.02	317	745	327	892	2.8	0.8	0.6	OR-AND-aTc	aTc gate
10	0.5	317	342	337	380	1.2	0.1	0.6	OR-AND-aTc	SLOPE gate
20	0.5	317	408	337	493	1.6	0.4	0.6	OR-AND-aTc	Asym AND gate
30	0.5	317	481	337	651	2.1	0.5	0.7	OR-AND-aTc	Asym AND gate
50	0.5	317	745	337	1426	4.5	0.5	0.7	OR-AND-aTc	Asym AND gate
10	1	317	342	332	395	1.2	0.1	0.7	OR-AND-aTc	SLOPE gate
20	1	317	408	332	510	1.6	0.4	0.7	OR-AND-aTc	Asym AND gate
30	1	317	481	332	686	2.2	0.5	0.7	OR-AND-aTc	Asym AND gate
50	1	317	745	332	1437	4.5	0.5	0.7	OR-AND-aTc	Asym AND gate

Supplementary Table S13: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG06. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	314	438	317	461	1.5	0.8	0.6	OR-AND-aTc	aTc gate
20	0.01	314	731	317	1082	3.4	0.7	0.7	OR-AND-aTc	Asym AND gate
30	0.01	314	1789	317	2610	8.3	0.8	0.6	OR-AND-aTc	aTc gate
50	0.01	314	3309	317	5085	16.2	0.8	0.6	OR-AND-aTc	aTc gate
10	0.02	314	438	330	482	1.5	0.7	0.6	OR-AND-aTc	Asym SLOPE gate
20	0.02	314	731	330	1431	4.6	0.5	0.7	OR-AND-aTc	Asym AND gate
30	0.02	314	1789	330	3010	9.6	0.7	0.6	OR-AND-aTc	Asym SLOPE gate
50	0.02	314	3309	330	7266	23.1	0.7	0.6	OR-AND-aTc	Asym SLOPE gate
10	0.5	314	438	324	565	1.8	0.5	0.7	OR-AND-aTc	Asym AND gate
20	0.5	314	731	324	1870	6.0	0.5	0.8	OR-AND-aTc	Asym AND gate
30	0.5	314	1789	324	4321	13.8	0.7	0.7	OR-AND-aTc	Asym AND gate
50	0.5	314	3309	324	11963	38.1	0.6	0.7	OR-AND-aTc	Asym AND gate
10	1	314	438	327	596	1.9	0.5	0.7	OR-AND-aTc	Asym AND gate
20	1	314	731	327	1725	5.5	0.5	0.7	OR-AND-aTc	Asym AND gate
30	1	314	1789	327	4788	15.2	0.6	0.7	OR-AND-aTc	Asym AND gate
50	1	314	3309	327	10201	32.5	0.7	0.7	OR-AND-aTc	Asym AND gate

Supplementary Table S14: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG07. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	313	382	318	380	1.2	0.9	0.5	OR-(a)N(I)-aTc	aTc gate
20	0.01	313	615	318	535	2.0	0.8	0.6	OR-(a)N(I)-aTc	aTc gate
30	0.01	313	1551	318	1626	5.2	1.0	0.5	OR-AND-aTc	aTc gate
50	0.01	313	3421	318	3968	12.7	0.9	0.5	OR-AND-aTc	aTc gate
10	0.02	313	382	322	392	1.3	0.8	0.5	OR-AND-aTc	aTc gate
20	0.02	313	615	322	646	2.1	0.9	0.5	OR-AND-aTc	aTc gate
30	0.02	313	1551	322	1692	5.4	0.9	0.5	OR-AND-aTc	aTc gate
50	0.02	313	3421	322	4177	13.4	0.9	0.5	OR-AND-aTc	aTc gate
10	0.5	313	382	338	407	1.3	0.5	0.5	OR-AND-aTc	Asym SLOPE gate
20	0.5	313	615	338	650	2.1	0.8	0.5	OR-AND-aTc	aTc gate
30	0.5	313	1551	338	1702	5.4	0.9	0.5	OR-AND-aTc	aTc gate
50	0.5	313	3421	338	4091	13.1	0.9	0.5	OR-AND-aTc	aTc gate
10	1	313	382	341	374	1.2	0.5	0.3	OR-(a)N(I)-aTc	Asym aTc gate
20	1	313	615	341	593	2.0	0.8	0.5	OR-(a)N(I)-aTc	aTc gate
30	1	313	1551	341	1663	5.3	0.9	0.5	OR-AND-aTc	aTc gate
50	1	313	3421	341	4966	15.9	0.8	0.6	OR-AND-aTc	aTc gate

Supplementary Table S15: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG13. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	329	540	312	816	2.6	0.5	0.7	(a)I(I)-AND-aTc	Asym AND gate
20	0.01	329	2005	312	2602	8.3	0.9	0.5	(a)I(I)-AND-aTc	aTc gate
30	0.01	329	3535	312	4010	12.9	0.9	0.5	(a)I(I)-AND-aTc	aTc gate
50	0.01	329	5872	312	7274	23.3	0.9	0.5	(a)I(I)-AND-aTc	aTc gate
10	0.02	329	540	322	837	2.6	0.5	0.7	(a)I(I)-AND-aTc	Asym AND gate
20	0.02	329	2005	322	2562	8.0	0.9	0.6	(a)I(I)-AND-aTc	aTc gate
30	0.02	329	3535	322	4259	13.2	0.9	0.5	(a)I(I)-AND-aTc	aTc gate
50	0.02	329	5872	322	8466	26.3	0.9	0.6	(a)I(I)-AND-aTc	aTc gate
10	0.5	329	540	331	655	2.0	0.7	0.6	OR-AND-aTc	Asym AND gate
20	0.5	329	2005	331	3379	10.3	0.8	0.6	OR-AND-aTc	aTc gate
30	0.5	329	3535	331	4961	15.1	0.9	0.6	OR-AND-aTc	aTc gate
50	0.5	329	5872	331	9889	30.1	0.8	0.6	OR-AND-aTc	aTc gate
10	1	329	540	352	423	1.6	0.4	0.7	OR-(a)N(I)-aTc	Asym (a)N(I) gate
20	1	329	2005	352	3389	10.3	0.7	0.6	OR-AND-aTc	Asym SLOPE gate
30	1	329	3535	352	6025	18.3	0.8	0.6	OR-AND-aTc	aTc gate
50	1	329	5872	352	10706	32.5	0.8	0.6	OR-AND-aTc	aTc gate

Supplementary Table S16: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG15. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	307	334	4086	4032	13.3	1.0	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.01	307	454	4086	4092	13.3	0.8	0.4	OR-AND-IPTG	IPTG gate
30	0.01	307	613	4086	3957	13.3	0.7	0.4	OR-(I)N(a)-IPTG	Asym IPTG gate
50	0.01	307	883	4086	6895	22.4	0.5	0.4	OR-AND-IPTG	Asym SLOPE gate
10	0.02	307	334	5994	6112	19.9	1.0	0.5	OR-AND-IPTG	IPTG gate
20	0.02	307	454	5994	6294	20.5	0.9	0.4	OR-AND-IPTG	IPTG gate
30	0.02	307	613	5994	6637	21.6	0.7	0.4	OR-AND-IPTG	Asym SLOPE gate
50	0.02	307	883	5994	10821	35.2	0.5	0.4	OR-AND-IPTG	Asym SLOPE gate
10	0.5	307	334	11816	11947	38.9	1.0	0.5	OR-AND-IPTG	IPTG gate
20	0.5	307	454	11816	12276	40.0	0.9	0.5	OR-AND-IPTG	IPTG gate
30	0.5	307	613	11816	14410	46.9	0.8	0.4	OR-AND-IPTG	IPTG gate
50	0.5	307	883	11816	22437	73.0	0.6	0.5	OR-AND-IPTG	Asym SLOPE gate
10	1	307	334	12415	12895	42.0	1.0	0.5	OR-AND-IPTG	IPTG gate
20	1	307	454	12415	12970	42.2	0.9	0.5	OR-AND-IPTG	IPTG gate
30	1	307	613	12415	15991	52.0	0.8	0.4	OR-AND-IPTG	IPTG gate
50	1	307	883	12415	24218	78.8	0.6	0.5	OR-AND-IPTG	Asym SLOPE gate

Supplementary Table S17: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG21. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	306	335	2561	2211	8.4	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.01	306	416	2561	2853	9.3	0.8	0.5	OR-AND-IPTG	IPTG gate
30	0.01	306	680	2561	3624	11.8	0.5	0.4	OR-AND-IPTG	Asym SLOPE gate
50	0.01	306	1262	2561	5336	17.4	0.2	0.4	OR-AND-IPTG	SLOPE gate
10	0.02	306	335	4011	3604	13.1	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.02	306	416	4011	4548	14.8	0.8	0.5	OR-AND-IPTG	IPTG gate
30	0.02	306	680	4011	5569	18.2	0.6	0.4	OR-AND-IPTG	Asym SLOPE gate
50	0.02	306	1262	4011	11375	37.1	0.3	0.4	OR-AND-IPTG	Asym SLOPE gate
10	0.5	306	335	8007	8987	29.3	0.9	0.5	OR-AND-IPTG	IPTG gate
20	0.5	306	416	8007	9070	29.6	0.9	0.5	OR-AND-IPTG	IPTG gate
30	0.5	306	680	8007	12126	39.6	0.7	0.4	OR-AND-IPTG	Asym SLOPE gate
50	0.5	306	1262	8007	22972	75.0	0.4	0.5	OR-AND-IPTG	Asym SLOPE gate
10	1	306	335	9651	8689	31.5	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	1	306	416	9651	9856	32.2	0.9	0.5	OR-AND-IPTG	IPTG gate
30	1	306	680	9651	12274	40.1	0.7	0.4	OR-AND-IPTG	Asym SLOPE gate
50	1	306	1262	9651	26493	86.5	0.5	0.5	OR-AND-IPTG	Asym SLOPE gate

Supplementary Table S18: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG23. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		Condition				Logic parameters				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	335	429	9786	8620	29.2	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.01	335	739	9786	8980	29.2	0.7	0.4	OR-(I)N(a)-IPTG	Asym SLOPE gate*
30	0.01	335	1440	9786	9995	29.8	0.6	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.01	335	3233	9786	9284	29.2	0.3	0.2	OR-(I)N(a)-IPTG	Asym IPTG gate
10	0.02	335	429	12128	10343	36.2	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.02	335	739	12128	13214	39.4	0.8	0.4	OR-AND-IPTG	IPTG gate
30	0.02	335	1440	12128	12045	36.2	0.6	0.3	OR-(I)N(a)-IPTG	Asym IPTG gate
50	0.02	335	3233	12128	14092	42.1	0.4	0.2	OR-AND-IPTG	Asym IPTG gate
10	0.5	335	429	15626	15733	47.0	0.9	0.5	OR-AND-IPTG	IPTG gate
20	0.5	335	739	15626	15998	47.7	0.8	0.4	OR-AND-IPTG	IPTG gate
30	0.5	335	1440	15626	23256	69.4	0.6	0.4	OR-AND-IPTG	Asym IPTG gate
50	0.5	335	3233	15626	49741	148.5	0.3	0.4	OR-AND-IPTG	Asym SLOPE gate
10	1	335	429	15679	15580	46.8	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	1	335	739	15679	16805	50.2	0.8	0.4	OR-AND-IPTG	IPTG gate
30	1	335	1440	15679	22992	68.6	0.6	0.4	OR-AND-IPTG	Asym IPTG gate
50	1	335	3233	15679	33779	100.8	0.3	0.3	OR-AND-IPTG	Asym IPTG gate

* Refers to the “asymmetric SLOPE (*a*=0.5, *l*=0.5) gate” in the OR-(a)N(I)-aTc space.

Supplementary Table S19: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG25. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	310	350	10053	10131	32.7	1.0	0.5	OR-AND-IPTG	IPTG gate
20	0.01	310	733	10053	9896	32.5	0.7	0.4	OR-(I)N(a)-IPTG	Asym SLOPE gate*
30	0.01	310	1490	10053	10883	35.1	0.5	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.01	310	3099	10053	11853	38.3	0.3	0.2	OR-AND-IPTG	Asym IPTG gate
10	0.02	310	350	12744	12666	41.1	1.0	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.02	310	733	12744	12988	41.9	0.8	0.4	OR-AND-IPTG	IPTG gate
30	0.02	310	1490	12744	13915	44.9	0.6	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.02	310	3099	12744	19675	63.5	0.3	0.3	OR-AND-IPTG	Asym IPTG gate
10	0.5	310	350	15299	15497	50.0	1.0	0.5	OR-AND-IPTG	IPTG gate
20	0.5	310	733	15299	14585	49.4	0.8	0.4	OR-(I)N(a)-IPTG	IPTG gate
30	0.5	310	1490	15299	16158	52.2	0.6	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.5	310	3099	15299	37193	120.1	0.3	0.4	OR-AND-IPTG	Asym IPTG gate
10	1	310	350	14976	15175	49.0	1.0	0.5	OR-AND-IPTG	IPTG gate
20	1	310	733	14976	15130	48.9	0.8	0.4	OR-AND-IPTG	IPTG gate
30	1	310	1490	14976	12350	48.4	0.5	0.3	OR-(I)N(a)-IPTG	Asym IPTG gate
50	1	310	3099	14976	39472	127.4	0.3	0.4	OR-AND-IPTG	Asym IPTG gate

* Refers to the “asymmetric SLOPE (*a*=0.5, *l*=0.5) gate” in the OR-(a)N(I)-aTc space.

Supplementary Table S20: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG26. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		Condition				Logic parameters				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	442	589	6579	7092	16.0	0.9	0.5	OR-AND-IPTG	IPTG gate
20	0.01	442	1032	6579	8314	18.8	0.6	0.4	OR-AND-IPTG	Asym SLOPE gate
30	0.01	442	2317	6579	9786	22.1	0.3	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.01	442	4199	6579	11760	26.6	0.1	0.2	OR-AND-IPTG	OR gate
10	0.02	442	589	9535	10211	23.1	0.9	0.5	OR-AND-IPTG	IPTG gate
20	0.02	442	1032	9535	11136	25.2	0.7	0.4	OR-AND-IPTG	Asym SLOPE gate
30	0.02	442	2317	9535	13293	30.1	0.4	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.02	442	4199	9535	17241	39.0	0.2	0.3	OR-AND-IPTG	Asym IPTG gate
10	0.5	442	589	13124	11903	29.7	0.9	0.5	OR-(I)N(a)-IPTG	IPTG gate
20	0.5	442	1032	13124	14534	32.9	0.7	0.4	OR-AND-IPTG	Asym SLOPE gate
30	0.5	442	2317	13124	16379	37.0	0.5	0.3	OR-AND-IPTG	Asym IPTG gate
50	0.5	442	4199	13124	20640	46.7	0.3	0.3	OR-AND-IPTG	Asym IPTG gate
10	1	442	589	11329	12450	28.2	0.9	0.5	OR-AND-IPTG	IPTG gate
20	1	442	1032	11329	13886	31.4	0.7	0.4	OR-AND-IPTG	Asym SLOPE gate
30	1	442	2317	11329	16564	37.5	0.4	0.3	OR-AND-IPTG	Asym IPTG gate
50	1	442	4199	11329	23767	53.7	0.2	0.3	OR-AND-IPTG	Asym IPTG gate

Supplementary Table S21: GFP expression data with the corresponding *a*, *l*, *r*, logic parameter space and logic behavior for construct pAE_LG27. Basal always corresponds to aTc=0 ng/mL and IPTG=0 mM.

Inducer concentrations		**Condition**				**Logic parameters**				
aTc (ng/mL)	**IPTG (mM)**	**Basal**	**aTc**	**IPTG**	**a+I**	***r***	***a***	***l***	**Logic Space**	**Behavior**
10	0.01	12343	12107	14293	14182	1.2	0.8	0.5	(a)I(I)-(I)N(a)-IPTG	IPTG gate
20	0.01	12343	12649	14293	14205	1.2	0.8	0.4	OR-(I)N(a)-IPTG	IPTG gate
30	0.01	12343	13949	14293	16594	1.3	0.1	0.5	OR-AND-IPTG	SLOPE gate
50	0.01	12343	16624	14293	19187	1.6	0.3	0.5	OR-AND-aTc	Asym SLOPE gate
10	0.02	12343	12107	14723	15221	1.3	0.8	0.5	(a)I(I)-AND-IPTG	IPTG gate
20	0.02	12343	12649	14723	15413	1.2	0.7	0.5	OR-AND-IPTG	Asym SLOPE gate
30	0.02	12343	13949	14723	16585	1.3	0.2	0.5	OR-AND-IPTG	SLOPE gate
50	0.02	12343	16624	14723	19623	1.6	0.3	0.5	OR-AND-aTc	Asym SLOPE gate
10	0.5	12343	12107	15369	15571	1.3	0.9	0.5	(a)I(I)-AND-IPTG	IPTG gate
20	0.5	12343	12649	15369	16212	1.3	0.7	0.6	OR-AND-IPTG	Asym SLOPE gate
30	0.5	12343	13949	15369	17490	1.4	0.3	0.5	OR-AND-IPTG	Asym SLOPE gate
50	0.5	12343	16624	15369	20170	1.6	0.2	0.5	OR-AND-aTc	SLOPE gate
10	1	12343	12107	15632	15438	1.3	0.9	0.5	(a)I(I)-(I)N(a)-IPTG	IPTG gate
20	1	12343	12649	15632	15804	1.3	0.9	0.5	OR-AND-IPTG	IPTG gate
30	1	12343	13949	15632	17289	1.4	0.3	0.5	OR-AND-IPTG	Asym SLOPE gate
50	1	12343	16624	15632	20313	1.6	0.1	0.5	OR-AND-aTc	SLOPE gate

Mathematical Modeling Parameters and AIC

Supplementary Table S22: Nomenclature table for AND and OR gate mathematical models. Literature-derived parameters held fixed for transfer function modelling and sources are denoted. Values are not given for fitted constants here but are given in Supplementary Figures S11-S18, S25-S30.

Parameter	**Value**	**Units**	**Source**	**Description**
[RNAP](Munro *et al*, 2016)	3000	nM	Literature(5)	Concentration of RNAP
[LacI](Munro *et al*, 2016)	100	nM	Literature(5)	Concentration of LacI
[TetR](Munro *et al*, 2016)	100	nM	Literature(5)	Concentration of TetR
$K_{a,IPTG:LacI}$(Chens *et al*, 1994)	6.7e-04	nM^{-1}	Literature(6)	Association constant of IPTG to LacI
$K_{a,TetR:TetO}$(Kamionka *et al*, 2004)	5.6	nM^{-1}	Literature(7)	Association constant of TetR to TetO
$K_{a,aTc:TetR}$	-	nM^{-1}	Fitted	Association constant of aTc to TetR
$K_{a,LacI:LacO}$	-	nM^{-1}	Fitted	Association constant of LacI to LacO
$K_{a,RNAP:pLac}$	-	nM^{-1}	Fitted	Association constant of RNAP to pLac
$K_{a,RNAP:pTet}$	-	nM^{-1}	Fitted	Association constant of RNAP to pTet
a_{pTet}	-	n/a	Fitted	Promoter weight for pLac in OR gate modeling
a_{pTet}	-	n/a	Fitted	Promoter weight for pTet in OR gate modeling
b	-	n/a	Fitted	Cooperativity weight
c	-	n/a	Fitted	Cooperativity threshold
k	-	n/a	Fitted	Translation constant
a	-	n/a	Fitted	AND gate readthrough constant

Supplementary Table S23: Fitted promoter weights for OR gate model equations 2 and 3. The reported values for each construct correspond to the values from the construct's best fitting model equation, determined by the AIC and reported in Supplementary Table S7.

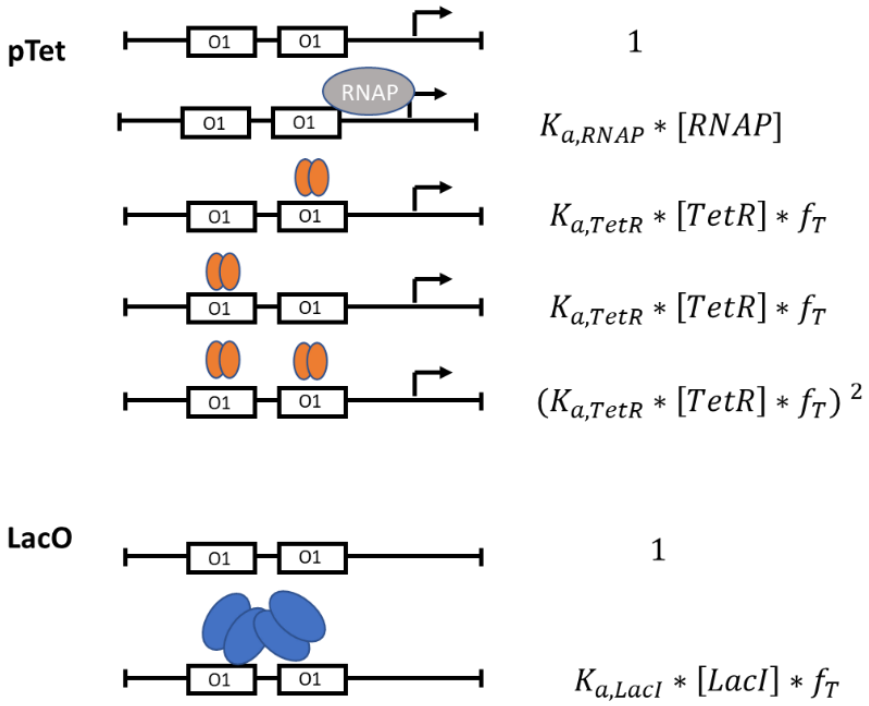
Construct	$\mathbf{a_{pTet}}$	$\mathbf{a_{pLac}}$
pAE_LG15	0.04	0.55
pAE_LG21	0.03	0.51
pAE_LG23	0.12	0.5
pAE_LG25	0.08	0.42
pAE_LG26	1.83	0.41
pAE_LG27	0.1	0.38

Supplementary Table S24: AIC values quantifying the goodness of fit for each model equation fit to a 5x5 grid of different aTc and IPTG concentrations for each AND construct. 'Hill' in the model equation name indicates a fitting of the transfer function Hill coefficients; model equations without 'Hill' have Hill equations set to 2.

	Construct name pAE_							
Model equation	**LG03**	**LG02**	**LG06**	**LG05**	**LG13**	**LG07**	**LG04**	**LG01**
Eqn S4	-54.37	-79.19	-69.44	-94.86	-73.33	-125.13	-86.82	-102.52
Eqn S4- Hill	-77.31	-75.52	-95.61	-142.71	-84.64	-125.28	-110.84	-96.70
Eqn S5	-46.41	-65.06	-69.44	-116.46	-71.43	-124.02	-86.23	-101.40
Eqn S5 Hill	-77.31	-76.08	-95.61	-139.98	-82.89	-123.57	-92.74	-96.70
Eqn S6- Hill	-74.10	-89.28	-126.98	-180.27	-96.53	-129.91	-99.59	-104.90
Eqn S7- Hill	-80.31	-110.29	-119.07	-175.87	-91.90	-126.90	-99.64	-97.97

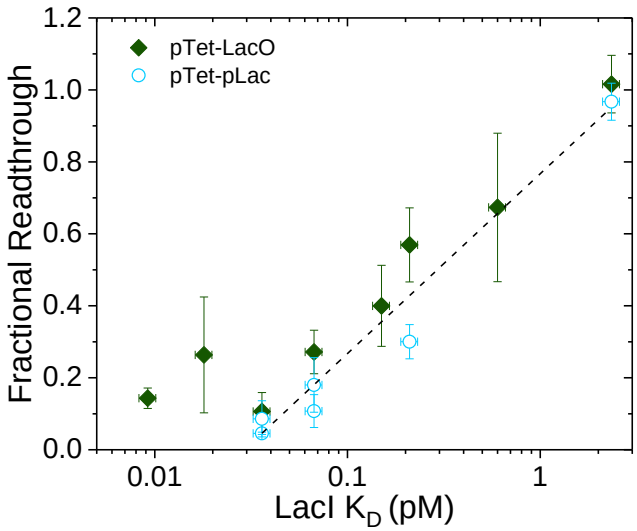
Supplementary Table S25: AIC values quantifying the goodness of fit for each model equation fit to a 5x5 grid of different aTc and IPTG concentrations for each OR construct. 'Hill' in the model equation name indicates a fitting of the transfer function Hill coefficients; model equations without 'Hill' have Hill parameters *m* and *n* set to 2.

	Construct name pAE_					
Model equation	**LG26**	**LG15**	**LG23**	**LG21**	**LG27**	**LG25**
Eqn S8	-32.72	67.19	44.19	67.57	66.28	43.95
Eqn S8- Hill	-10.13	13.94	-7.01	12.09	-43.92	2.98
Eqn S9	-84.30	-67.00	-49.36	-62.65	-111.02	-55.97
Eqn S9- Hill	-75.20	-64.20	-	-60.32	-112.75	-50.58
Eqn S10- Hill	-67.67	-68.13	-	-69.33	-110.99	-51.70



Supplementary Figure S1:

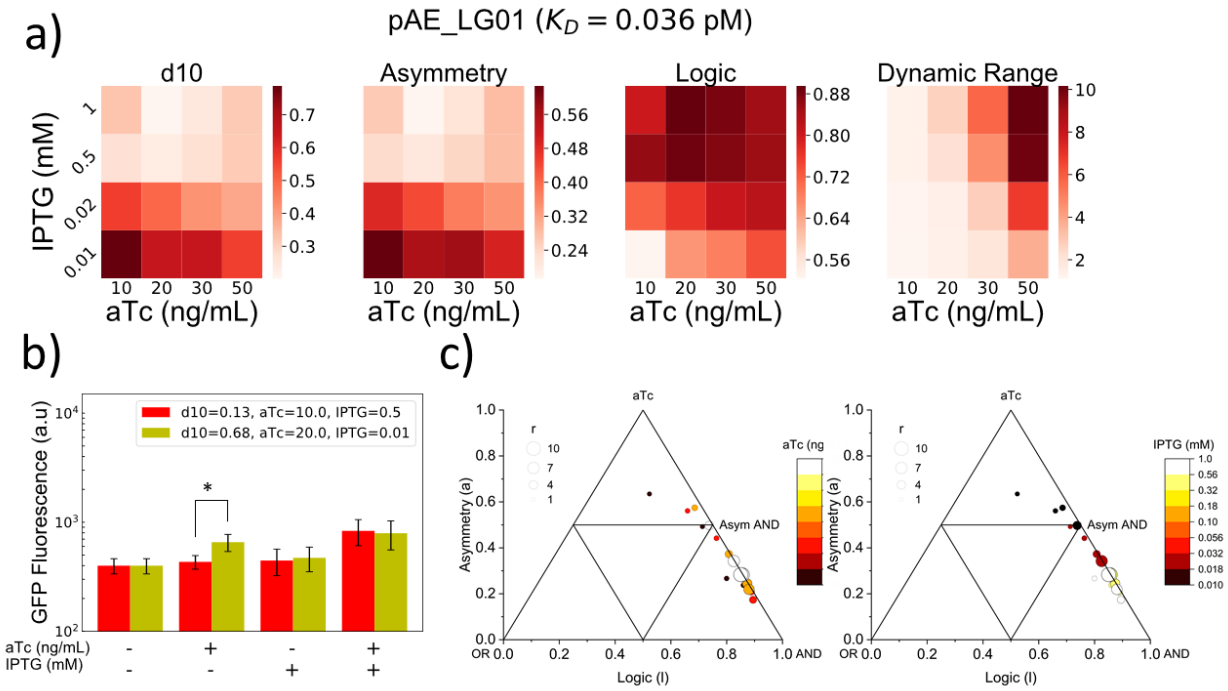
Graphical representation of pTet and LacO occupancy partitioning, adapted from Tamsir *et al* (Tamsir *et al*, 2011). The mathematical expressions representing each possible promoter state, shown on the right, are assembled into transfer functions, as shown in Equation S3.



Supplementary Figure S2:

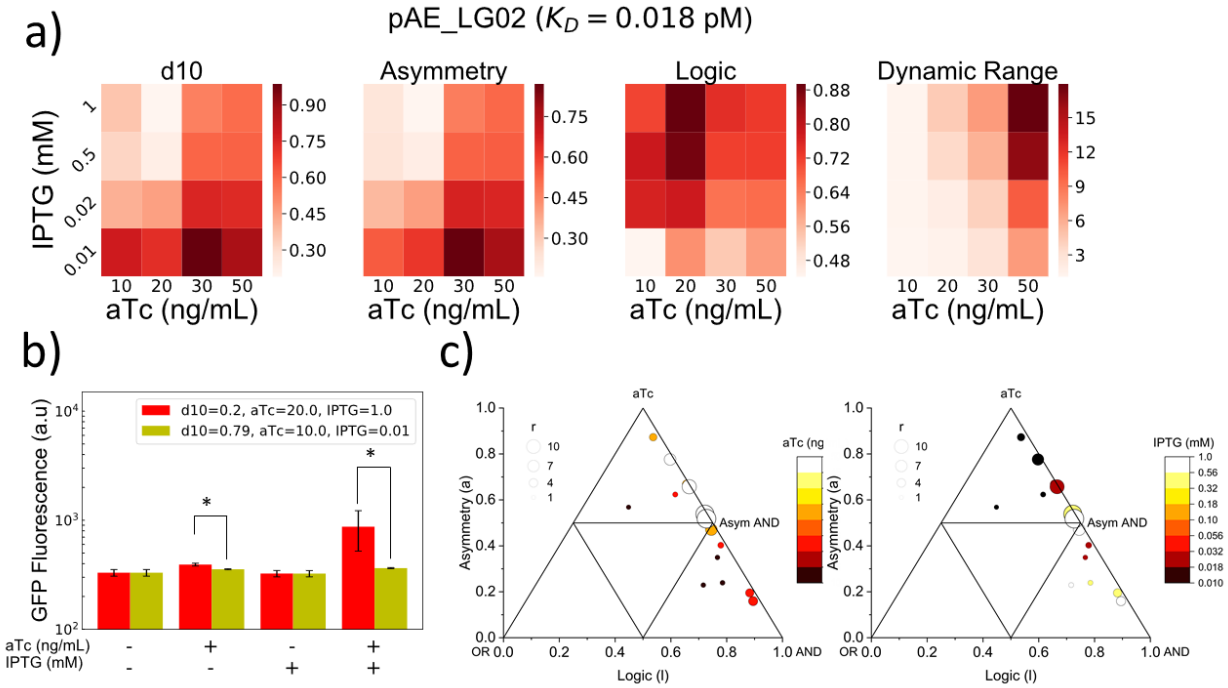
The fractional readthrough for constructs designed as OR gates (blue circles) is similar to that of constructs designed as AND gates (green rhombi). In fact, when fitted together for LacI K_D values greater than ~0.03 pM, a good correlation is obtained (Adj. R^2=0.97). This suggests that the main mode of TI in our set of tested tandem promoters is also roadblock, as it was in the case when a LacO was placed downstream of pTet.

AND Gate Logic Behaviors



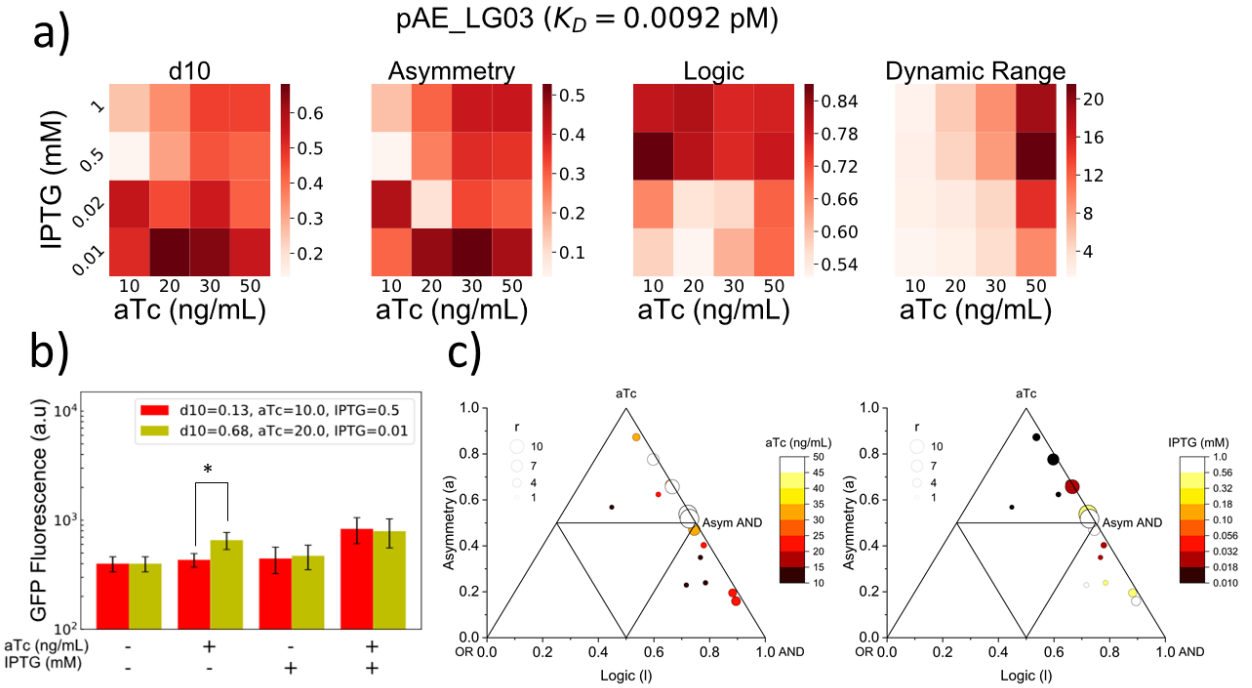
Supplementary Figure S3:

a) Heat maps of logic parameters of pAE_LG01. b) Bar plot of minimum and maximum d10 conditions for pAE_LG01. c) Triangle plots of pAE_LG01 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



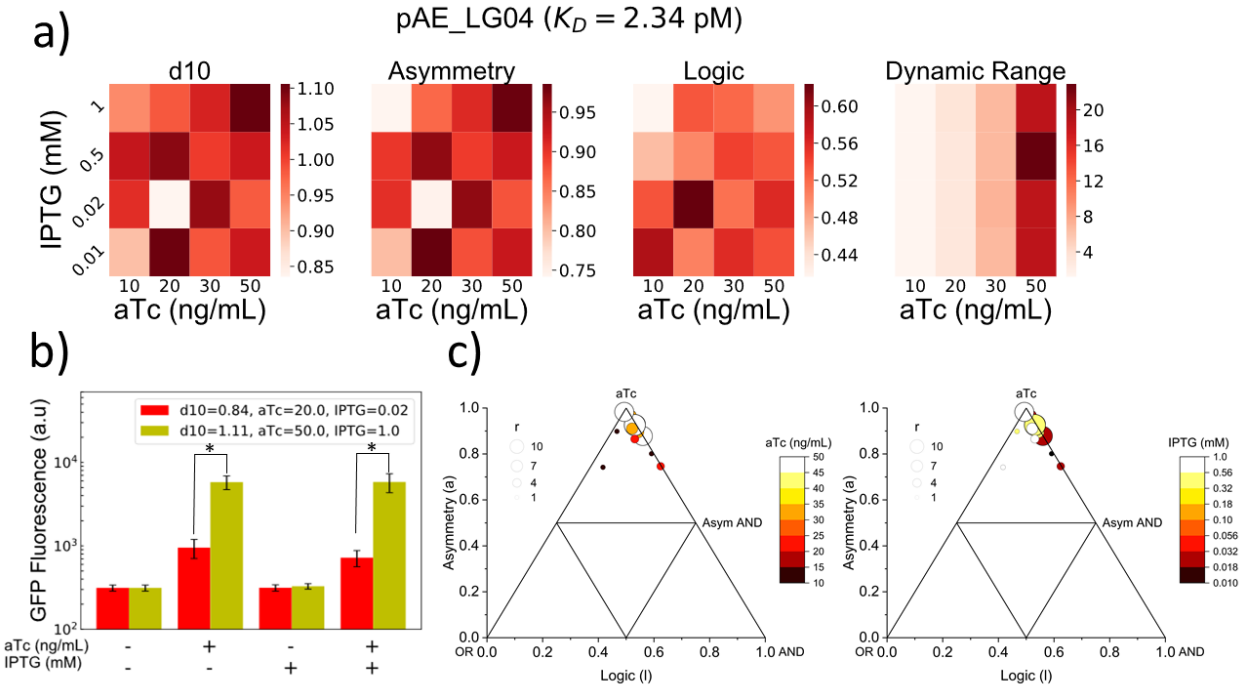
Supplementary Figure S4:

a) Heat maps of logic parameters of pAE_LG02. b) Bar plot of minimum and maximum d10 conditions for pAE_LG02. c) Triangle plots of pAE_LG02 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



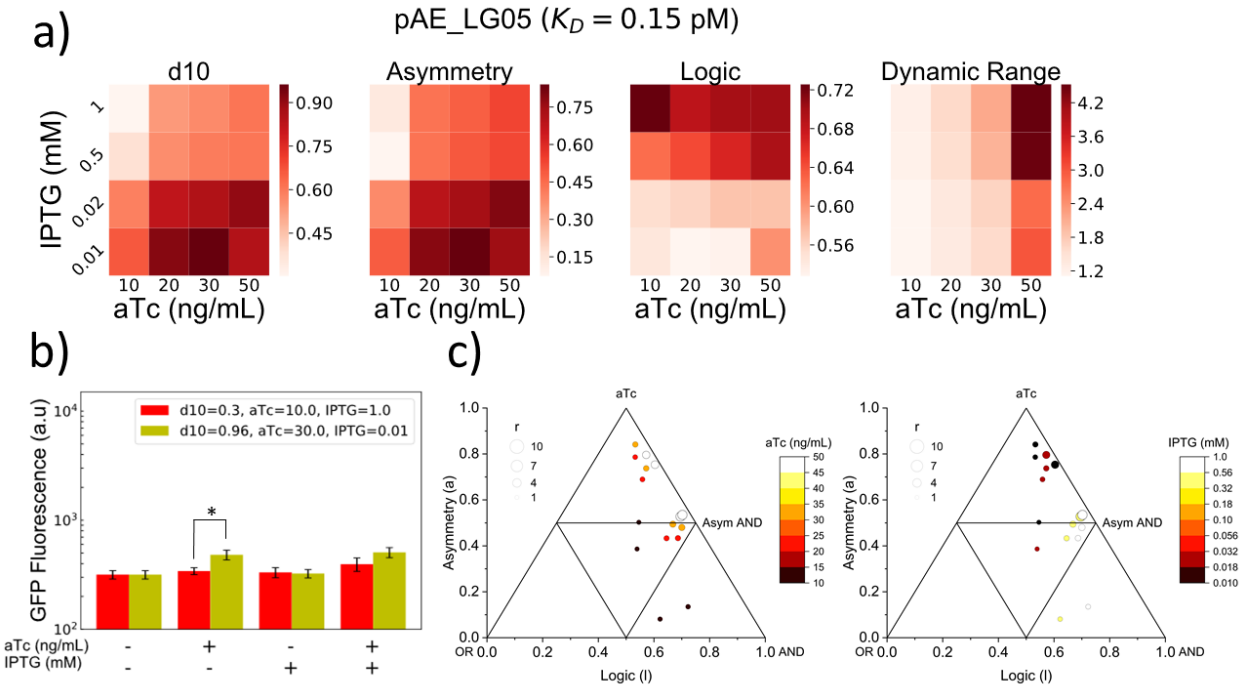
Supplementary Figure S5:

a) Heat maps of logic parameters of pAE_LG03. b) Bar plot of minimum and maximum d10 conditions for pAE_LG03. c) Triangle plots of pAE_LG03 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



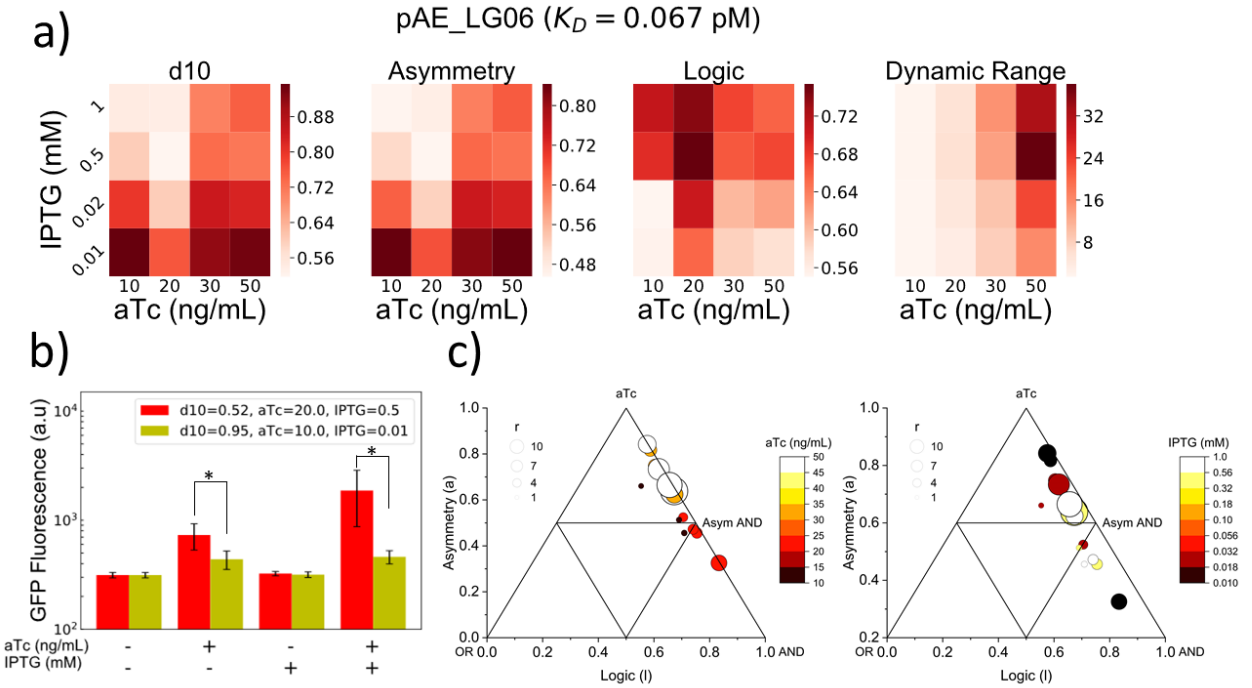
Supplementary Figure S6:

a) Heat maps of logic parameters of pAE_LG04. b) Bar plot of minimum and maximum d10 conditions for pAE_LG04. c) Triangle plots of pAE_LG04 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



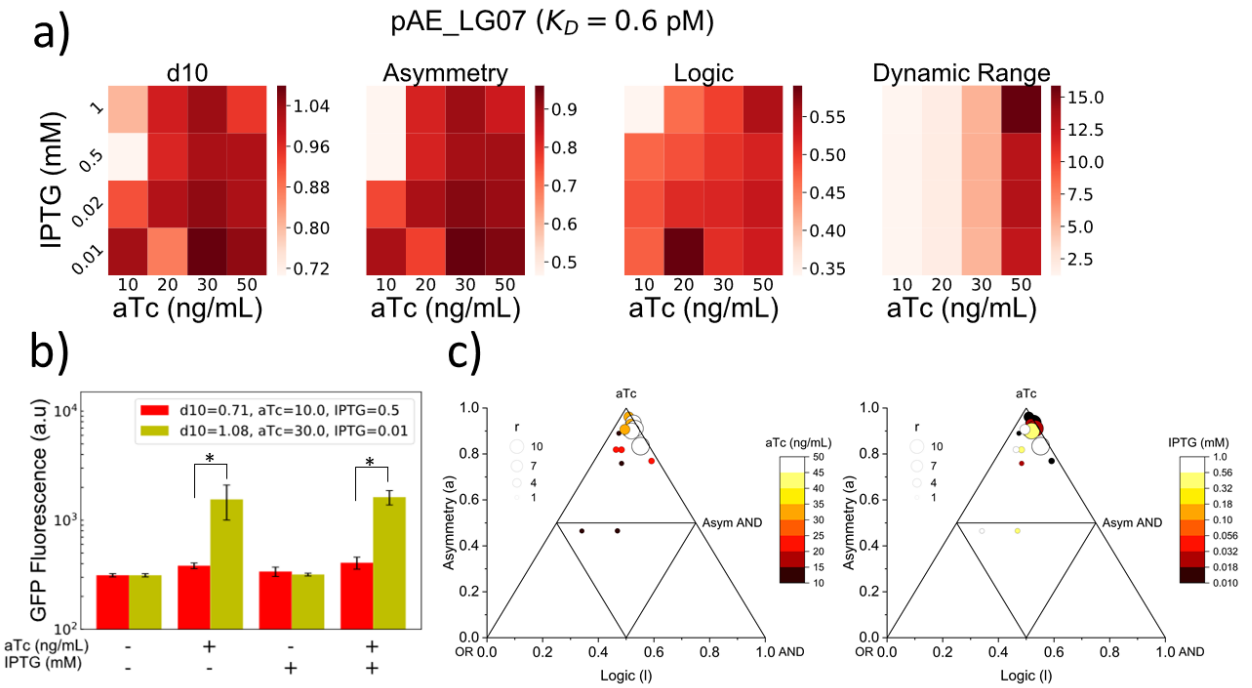
Supplementary Figure S7:

a) Heat maps of logic parameters of pAE_LG05. b) Bar plot of minimum and maximum d10 conditions for pAE_LG05. c) Triangle plots of pAE_LG05 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



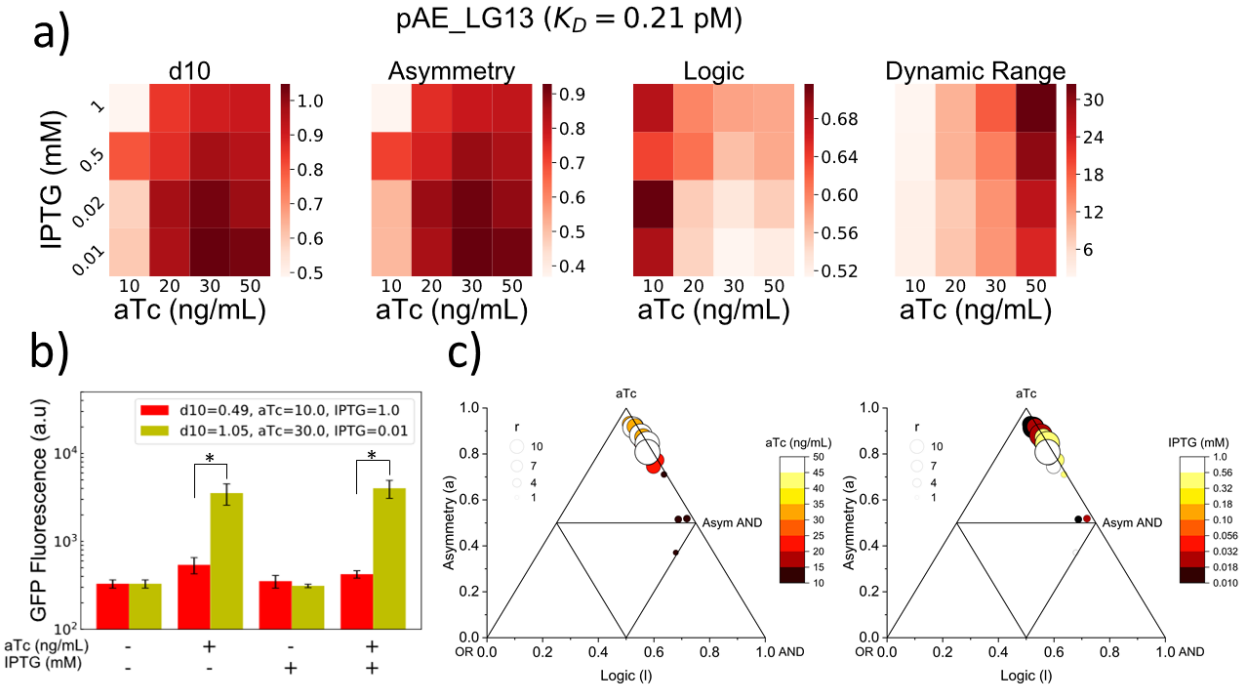
Supplementary Figure S8:

a) Heat maps of logic parameters of pAE_LG06. b) Bar plot of minimum and maximum d10 conditions for pAE_LG06. c) Triangle plots of pAE_LG06 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



Supplementary Figure S9:

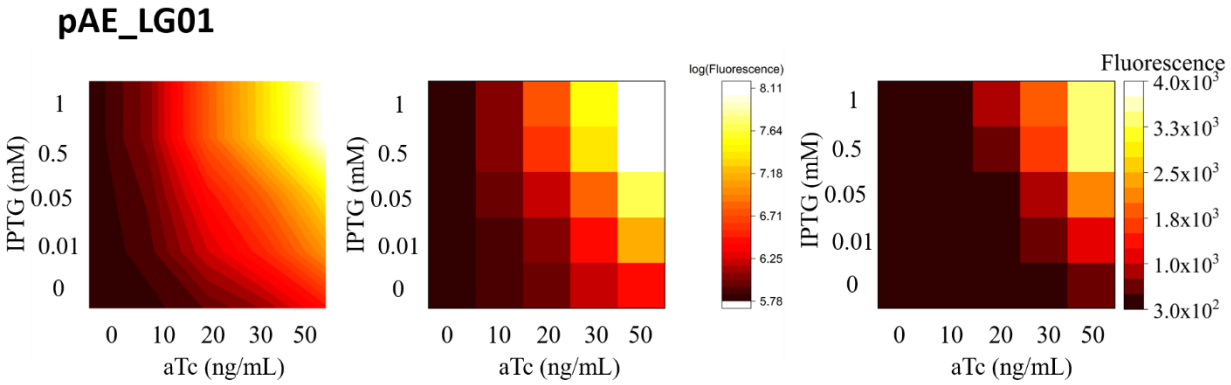
a) Heat maps of logic parameters of pAE_LG07. b) Bar plot of minimum and maximum d10 conditions for pAE_LG07. c) Triangle plots of pAE_LG07 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



Supplementary Figure S10:

a) Heat maps of logic parameters of pAE_LG13. b) Bar plot of minimum and maximum d10 conditions for pAE_LG13. c) Triangle plots of pAE_LG13 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).

AND Gate Model Fits



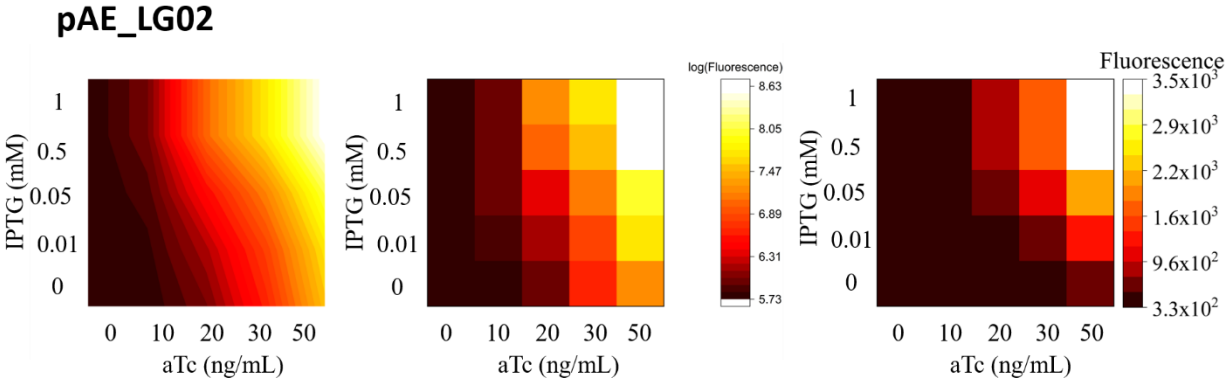
	value	+/-
K_aTcbind	1.00	(at bound)
K_LacIbind	0.22	0.15
K_RNAPbind	0.00	0.00
k_translation	12.00	(at bound)
m_TetRHill	1.79	0.10
n_LacIHill	1.34	0.14
a_Readthrough	-	-

AIC	-104.90
BIC	-100.03
RMSE	0.11
R^2	0.98

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	22	55.9	351	806	731
0.5	21.5	69.2	220	444	816
0.02	21.6	37.2	106	233	691
0.01	21.6	4.58	55.9	97.4	254
0	23.1	3.74	11.4	98.2	177
aTc	0	10	20	30	50

Supplementary Figure S11:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG01.



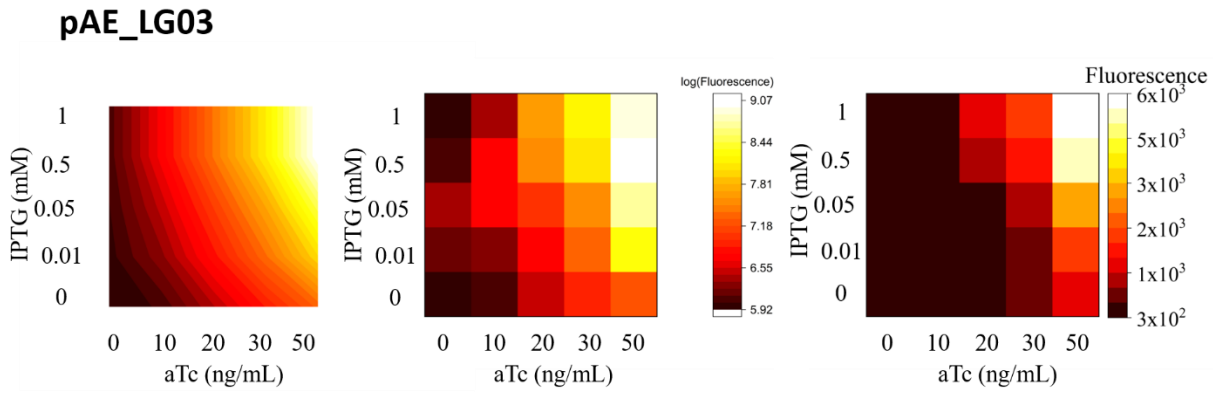
	value	+/-
K_aTcbind	0.14	0.29
K_LacIbind	3.42	7.48
K_RNAPbind	0.00	0.04
k_translation	3.58	3.47
m_TetRHill	4.00	3.23
n_LacIHill	2.24	0.89
a_Readthrough	0.33	0.07

AIC	-110.29
BIC	-101.76
RMSE	0.10
R^2	0.99

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	20	51	714	769	1979
0.5	21	64	689	623	1572
0.02	22	40	163	270	634
0.01	31	21	73	127	375
0	21	39	39	271	296
aTc	0	10	20	30	50

Supplementary Figure S12:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG02.



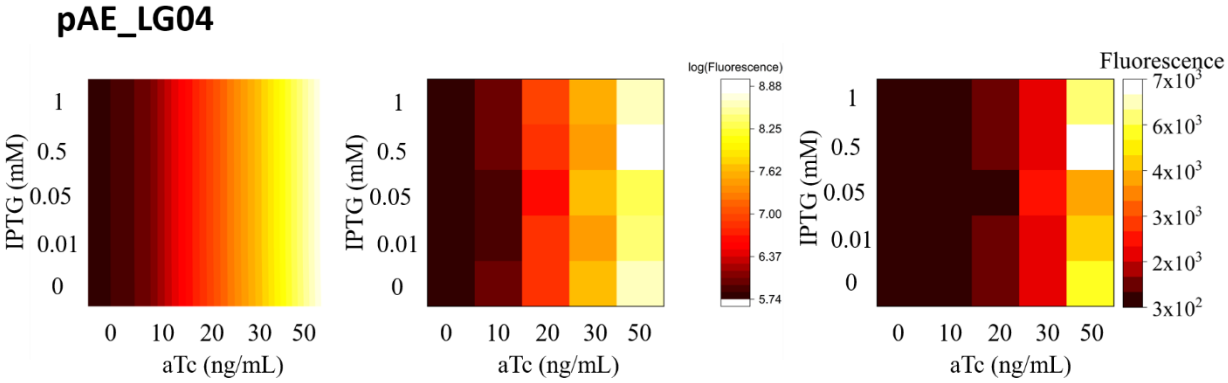
	value	+/-
K_aTcbind	0.06	0.09
K_LacIbind	0.33	0.63
K_RNAPbind	1.56	6.00
k_translation	12.00	34.38
m_TetRHill	0.54	0.49
n_LacIHill	1.63	0.84
a_Readthrough	0.87	0.37

AIC	-80.31
BIC	-71.78
RMSE	0.18
R^2	0.97

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	41.3	77.3	786	1142	1487
0.5	122	223	844	1004	4676
0.02	183	39.5	388	231	1937
0.01	120	110	235	595	1651
0	63.6	62.1	117	144	57.2
aTc	0	10	20	30	50

Supplementary Figure S13:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG03.



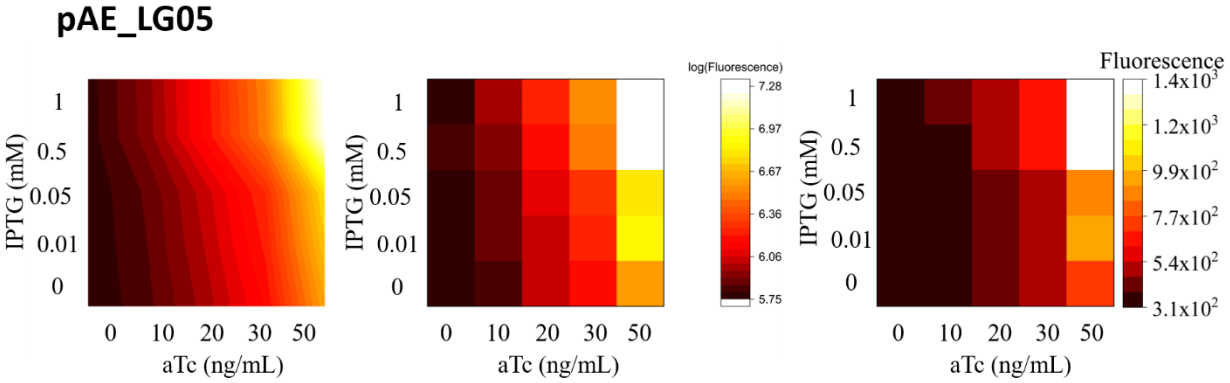
	value	+/-
K_aTcbind	0.82	0.52
K_LacIbind	0.00	At bound
K_RNAPbind	0.00	At bound
k_translation	5.51	-4.61
m_TetRHill	1.51	0.30
n_LacIHill	0.26	8.26
a_Readthrough	-	-

AIC	-110.84
BIC	-105.96
RMSE	0.10
R^2	0.99

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	24	28	536	547	1499
0.5	27	47	332	431	3659
0.02	27	44	158	1189	736
0.01	26	29	439	254	775
0	26	33	246	462	1073
aTc	0	10	20	30	50

Supplementary Figure S14:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG04.



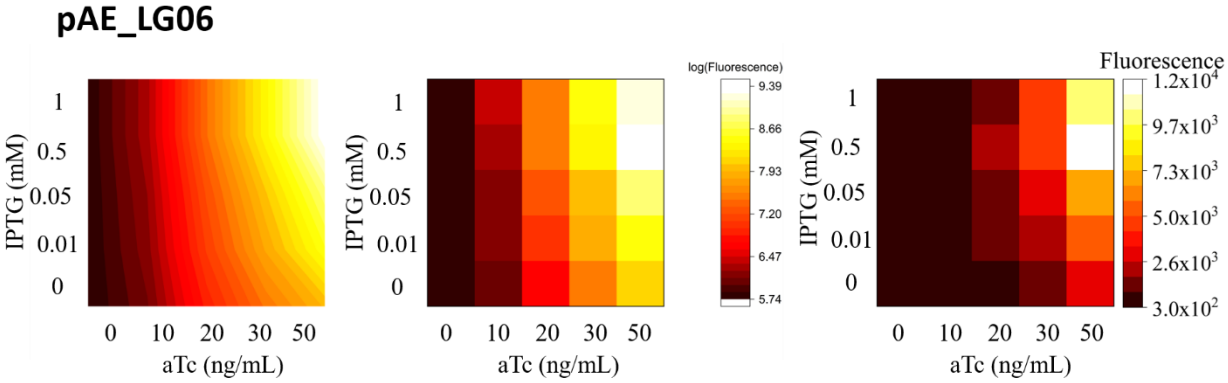
	value	+/-
K_aTcbind	0.05	0.03
K_LacIbind	0.43	0.32
K_RNAPbind	0.87	1.12
k_translation	2.83	2.62
m_TetRHill	0.98	0.17
n_LacIHill	1.06	0.26
a_Readthrough	-	-

AIC	-180.27
BIC	-172.95
RMSE	0.02
R^2	1.00

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	35	55	94	141	499
0.5	42	43	88	105	501
0.02	32	32	62	44	153
0.01	29	28	57	53	260
0	28	25	49	49	174
aTc	0	10	20	30	50

Supplementary Figure S15:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG05.



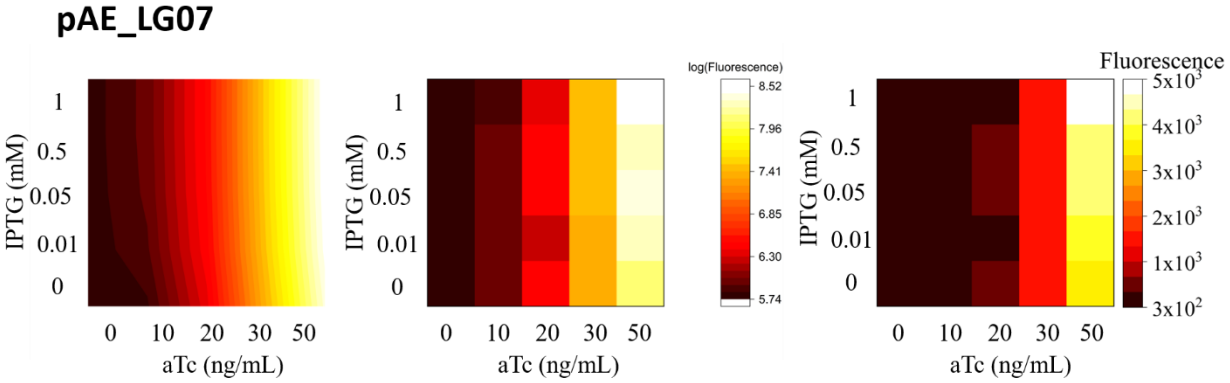
	value	+/-
K_aTcbind	0.12	0.11
K_LacIbind	0.20	0.16
K_RNAPbind	0.88	1.46
k_translation	2.39	0.18
m_TetRHill	1.02	0.10
n_LacIHill	1.39	0.29
a_Readthrough	-	-

AIC	-126.98
BIC	-119.67
RMSE	0.07
R^2	1.00

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	19	253	872	2340	3389
0.5	15	157	996	2193	5833
0.02	25	61	646	1339	3720
0.01	19	64	476	1014	2290
0	18	84	197	538	437
aTc	0	10	20	30	50

Supplementary Figure S16:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG06.



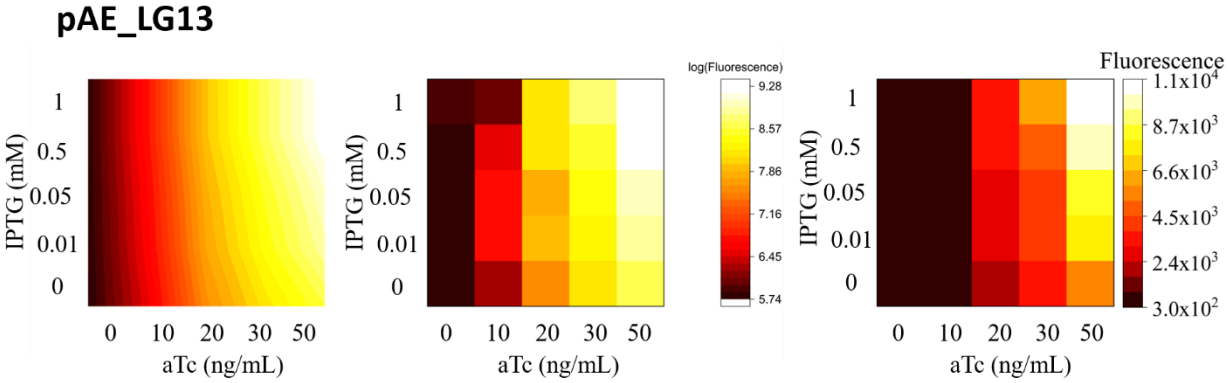
	value	+/-
K_aTcbind	0.04	0.09
K_LacIbind	0.44	0.63
K_RNAPbind	1.42	6.00
k_translation	2.64	34.38
m_TetRHill	0.01	0.49
n_LacIHill	2.34	0.84
a_Readthrough	-	-

AIC	-129.91
BIC	-123.81
RMSE	0.07
R^2	1.00

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	35	14	225	360	1195
0.5	33	51	310	644	797
0.02	11	35	179	480	175
0.01	9	20	89	245	133
0	10	22	161	548	175
aTc	0	10	20	30	50

Supplementary Figure S17:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG07.



	value	+/-
K_aTcbind	0.17	0.09
K_LacIbind	0.12	0.63
K_RNAPbind	1.07	6.00
k_translation	3.00	34.38
m_TetRHill	0.54	0.49
n_LacIHill	1.23	0.84
a_Readthrough	-	-

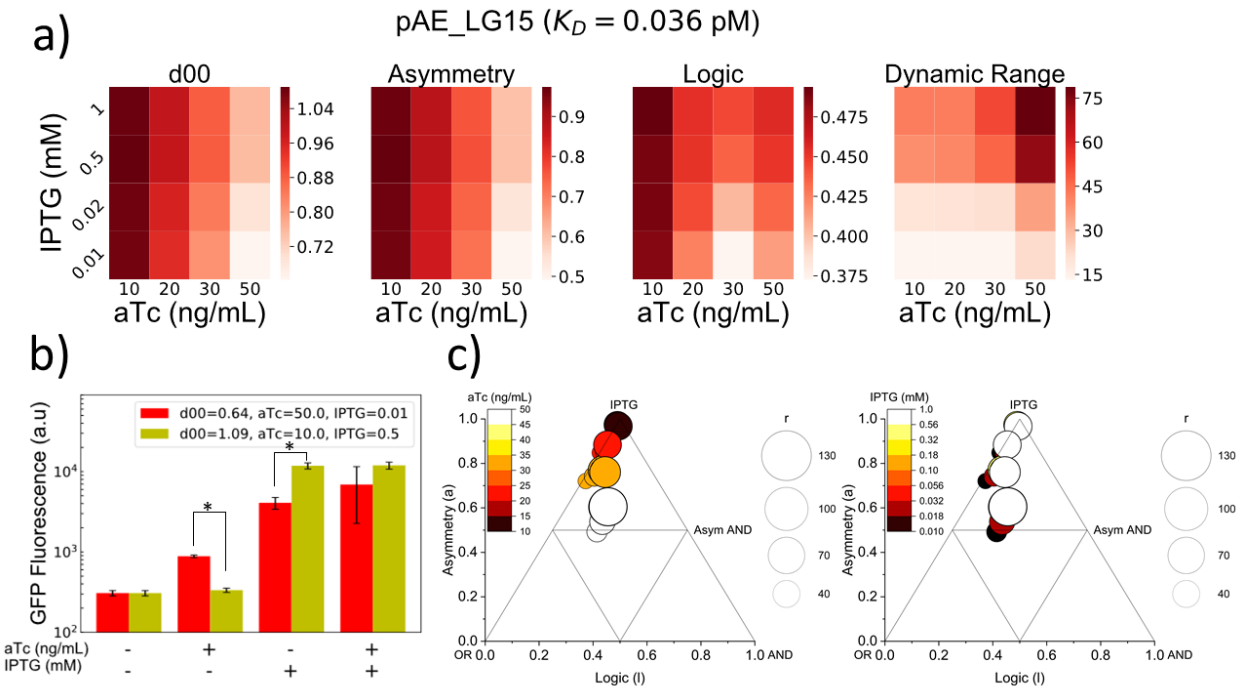
AIC	-96.53
BIC	-89.21
RMSE	0.13
R^2	0.99

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	58	40	1249	755	3372
0.5	31	325	1139	1694	2276
0.02	20	459	549	1074	1850
0.01	14	438	765	925	1666
0	35	114	330	959	1046
aTc	0	10	20	30	50

Supplementary Figure S18:

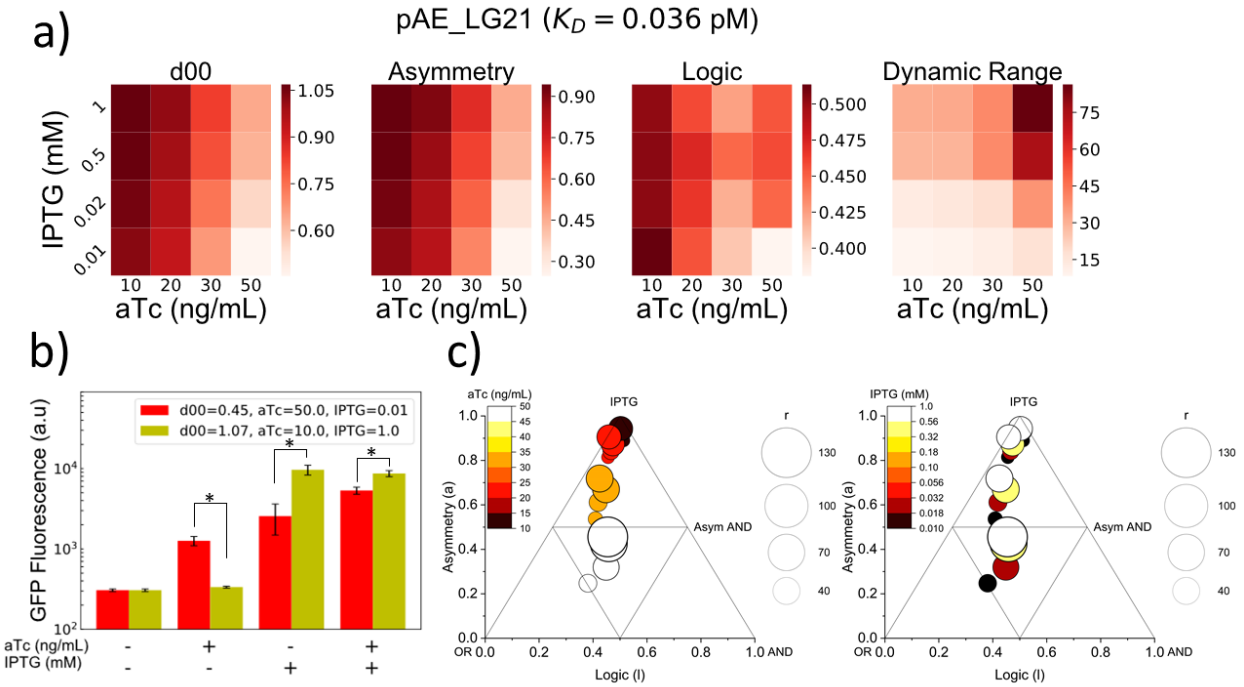
Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for AND gate pAE_LG13.

OR Gate Logic Behaviors



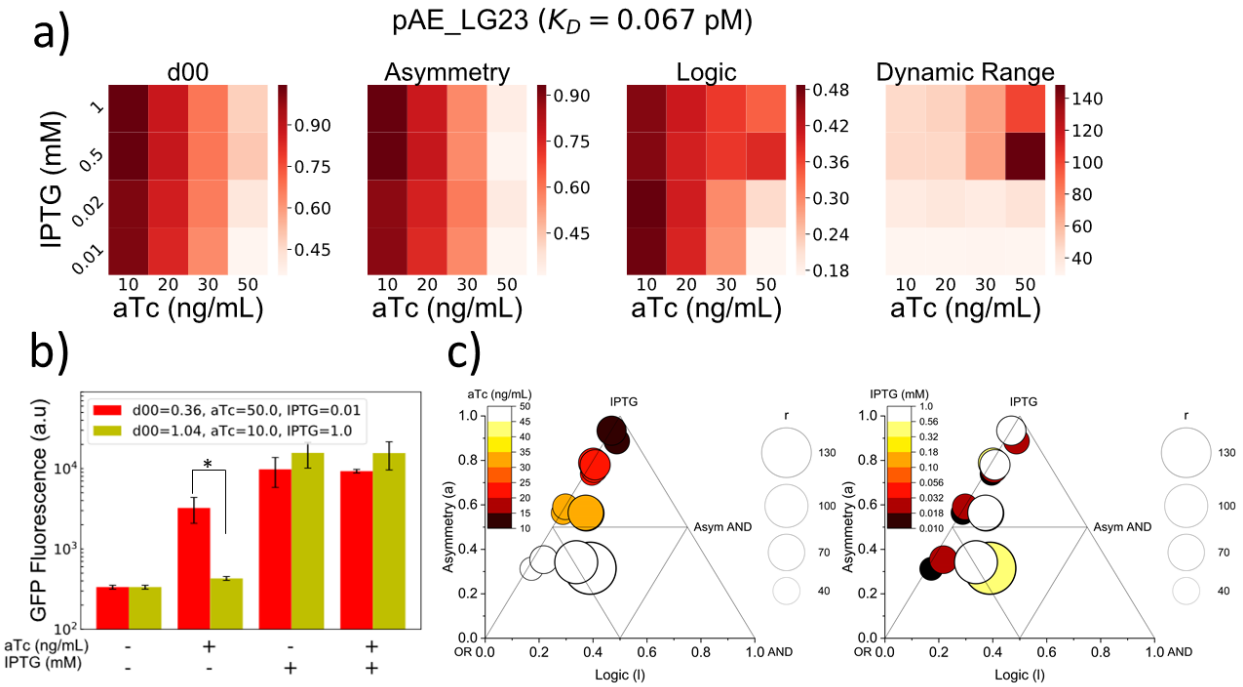
Supplementary Figure S19:

a) Heat maps of logic parameters of pAE_LG15. b) Bar plot of minimum and maximum d10 conditions for pAE_LG15. c) Triangle plots of pAE_LG15 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



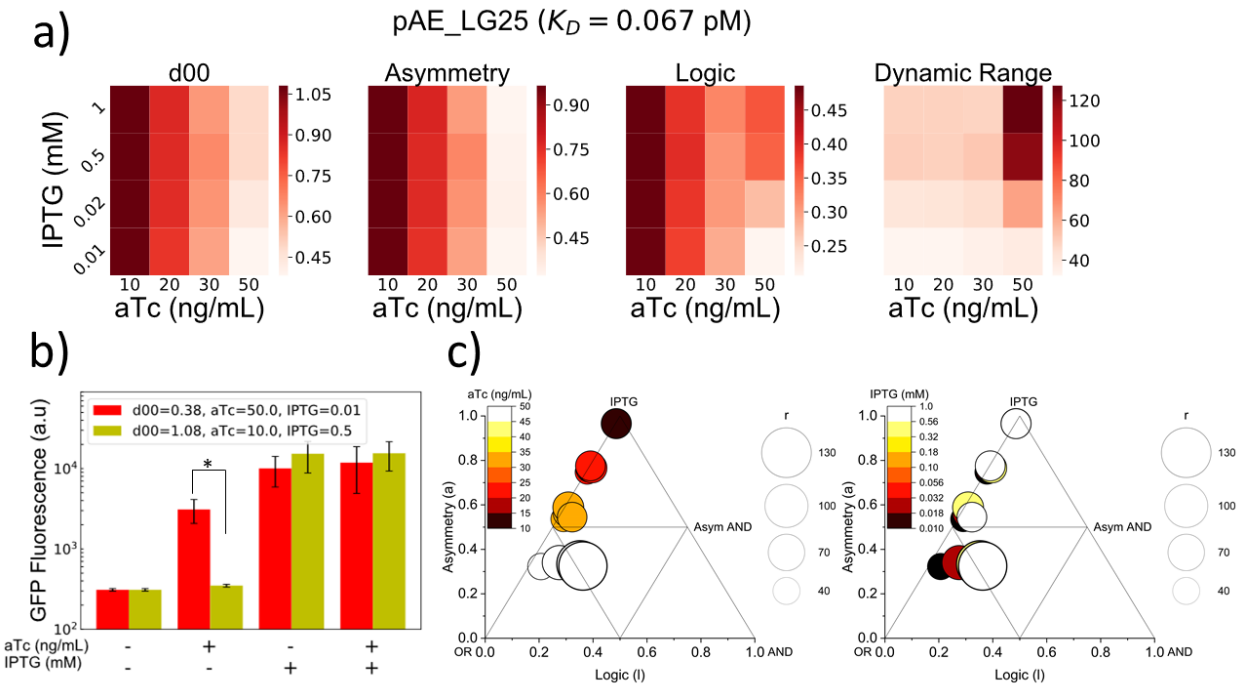
Supplementary Figure S20:

a) Heat maps of logic parameters of pAE_LG21. b) Bar plot of minimum and maximum d10 conditions for pAE_LG21. c) Triangle plots of pAE_LG21 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



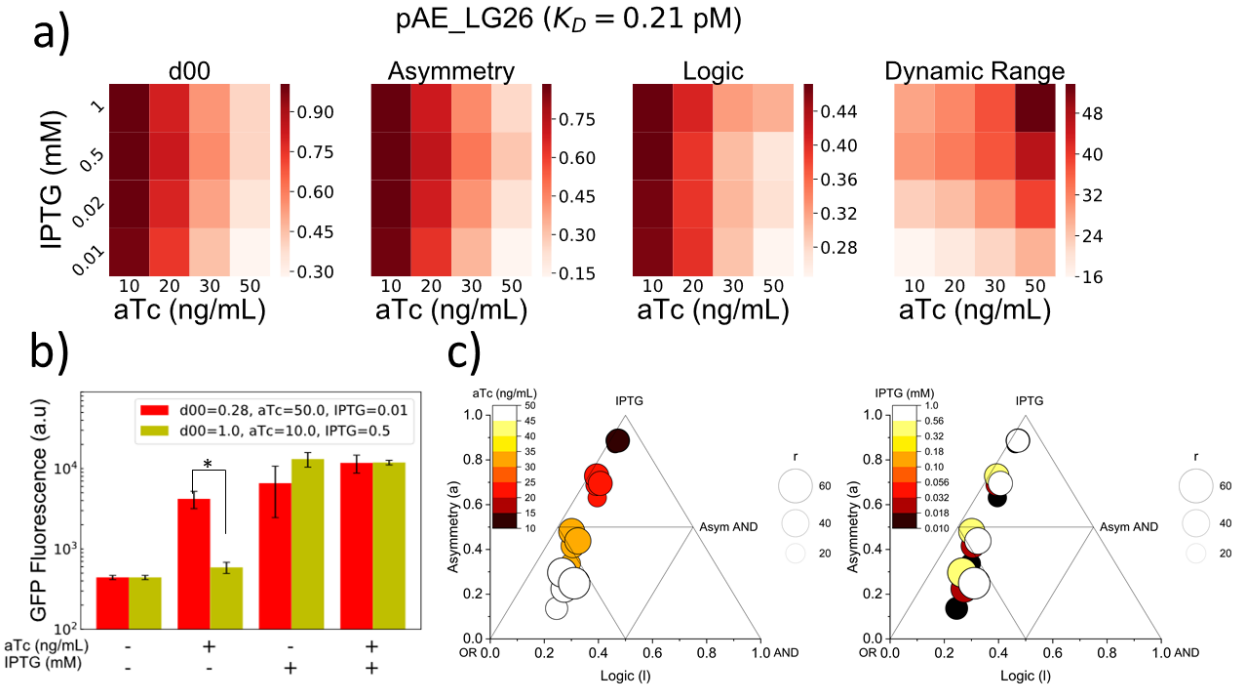
Supplementary Figure S21:

a) Heat maps of logic parameters of pAE_LG23. b) Bar plot of minimum and maximum d10 conditions for pAE_LG23. c) Triangle plots of pAE_LG23 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



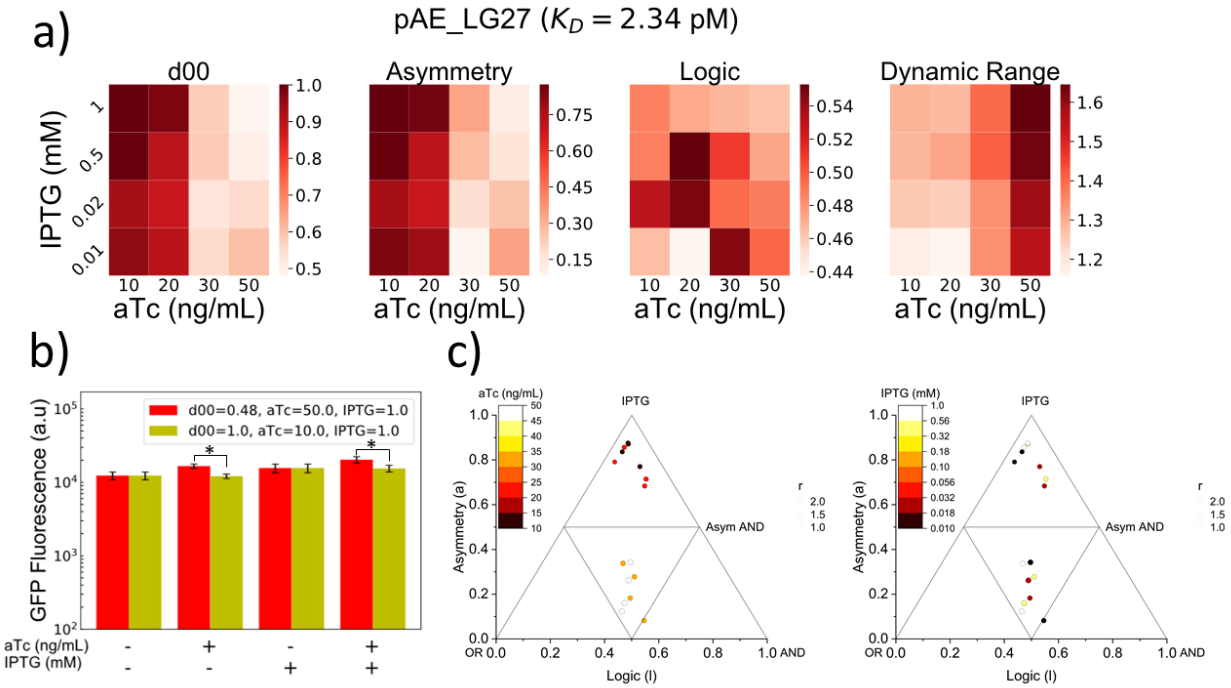
Supplementary Figure S22:

a) Heat maps of logic parameters of pAE_LG25. b) Bar plot of minimum and maximum d10 conditions for pAE_LG25. c) Triangle plots of pAE_LG25 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



Supplementary Figure S23:

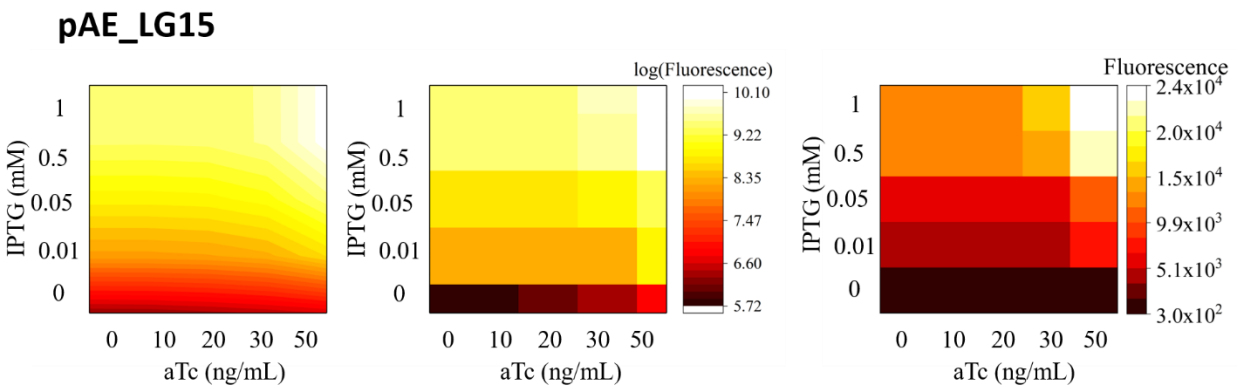
a) Heat maps of logic parameters of pAE_LG26. b) Bar plot of minimum and maximum d10 conditions for pAE_LG26. c) Triangle plots of pAE_LG26 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).



Supplementary Figure S24:

a) Heat maps of logic parameters of pAE_LG27. b) Bar plot of minimum and maximum d10 conditions for pAE_LG27. c) Triangle plots of pAE_LG27 at varying aTc and IPTG conditions, showing trends in aTc (left) and IPTG (right).

OR Gate Model Fits



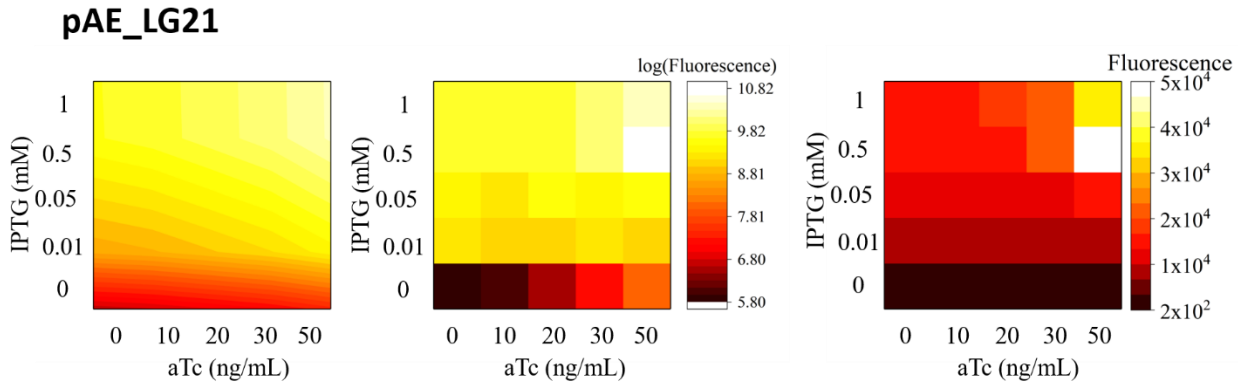
	value	+/-
RNAPbind2	6.15E-04	1.20E-03
a1	0.03	0.11
a2	0.55	0.38
m	1.95	8.69
n	1.68	0.61
b	1.90E-04	0.00
crit	75.83	78.07

AIC	-68.13
BIC	-59.60
R^2	0.98
RMSE	0.23

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	998	1045	884	1507	1366
0.5	1011	1188	1101	1392	2353
0.02	611	775	722	541	1003
0.01	684	528	117	454	4627
0	23	21	102	80	33
aTc	0	10	20	30	50

Supplementary Figure S25:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for OR gate pAE_LG15.



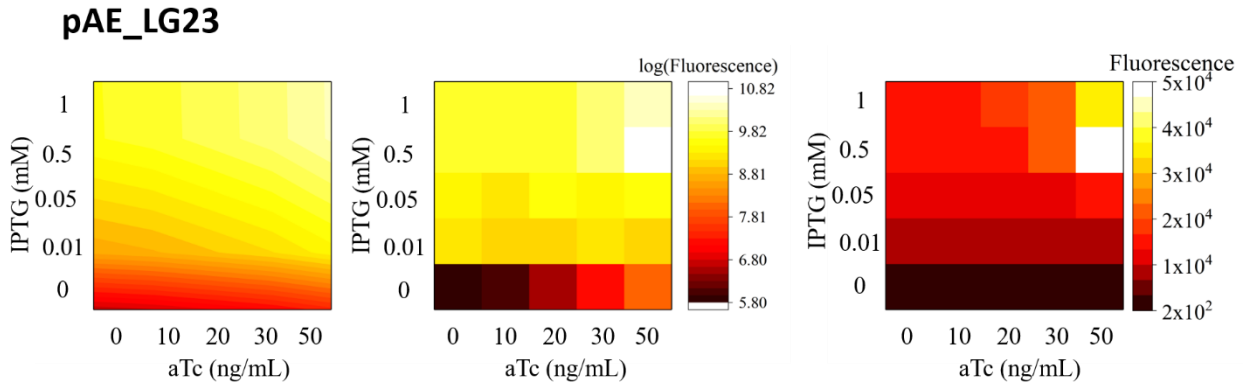
	value	+/-
RNAPbind2	5.39E-04	1.10E-03
a1	0.03	0.06
a2	0.51	0.41
m	2.53	6.25
n	1.59	0.59
b	2.54E-04	0.00
crit	50.35	81.18

AIC	-69.33
BIC	-60.80
R^2	0.98
RMSE	0.22

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	1355	744	683	780	7573
0.5	1087	1907	656	1213	6382
0.02	1072	741	755	432	2534
0.01	1071	258	764	1368	537
0	12	9	2	115	166
aTc	0	10	20	30	50

Supplementary Figure S26:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for OR gate pAE_LG21.



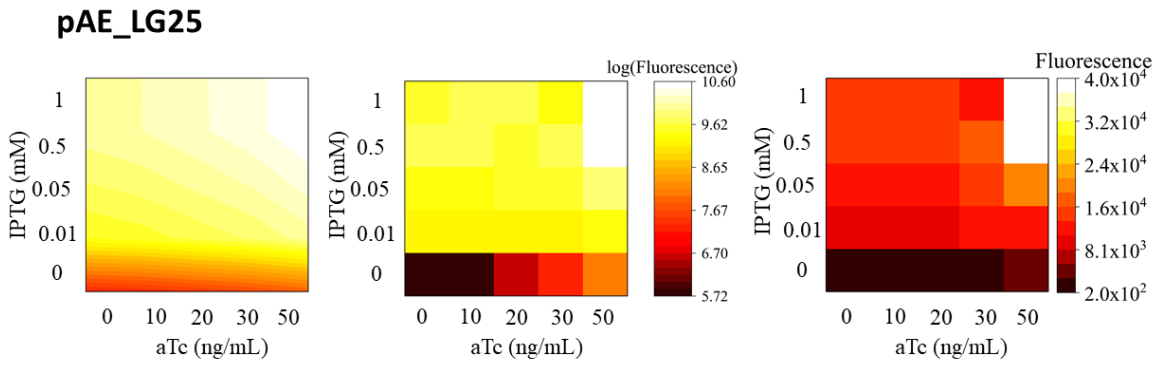
	value	+/-
RNAPbind2	1.01E-03	5.95E-04
a1	0.12	0.06
a2	0.50	0.09
m		
n		
b		
crit		

AIC	-49.36
BIC	-45.70
R^2	0.93
RMSE	0.35

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	5552	5950	5400	11490	24286
0.5	5968	7136	9747	12149	40875
0.02	4718	4964	5632	3943	2599
0.01	3939	3037	5278	5933	481
0	19	24	189	385	1148
aTc	0	10	20	30	50

Supplementary Figure S27:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for OR gate pAE_LG23.



	value	+/-
K_aTcbind	0.12	0.11
K_LacIbind	0.20	0.16
K_RNAPbind	0.88	1.46
k	2.39	0.18
m	1.02	0.10
n	1.39	0.29
RNAPbind2	2.55E-03	1.15E-03

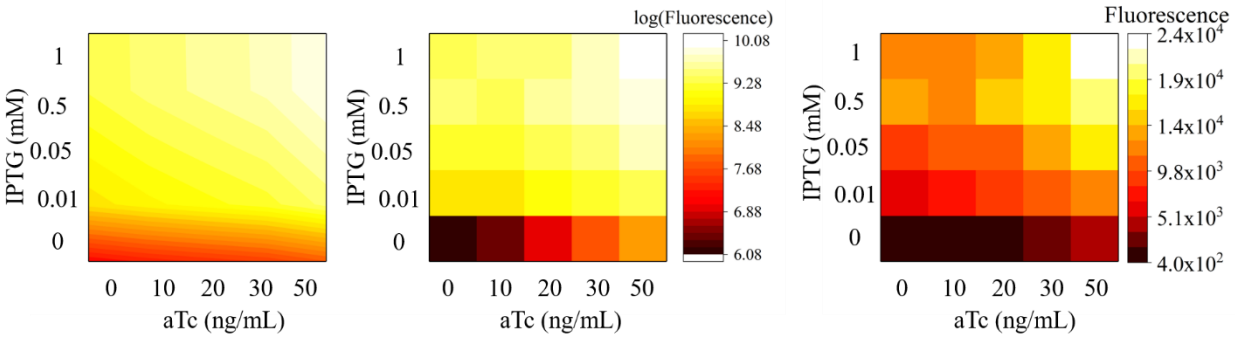
AIC	-55.97
BIC	-52.31
R^2	0.95
RMSE	0.31

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	7408	6157	3883	258	20269
0.5	6505	6169	4218	6115	18166
0.02	5936	5504	4984	4458	8327
0.01	4155	3838	3552	3765	6932
0	11	14	176	439	1018
aTc	0	10	20	30	50

Supplementary Figure S28:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for OR gate pAE_LG25.

pAE_LG26



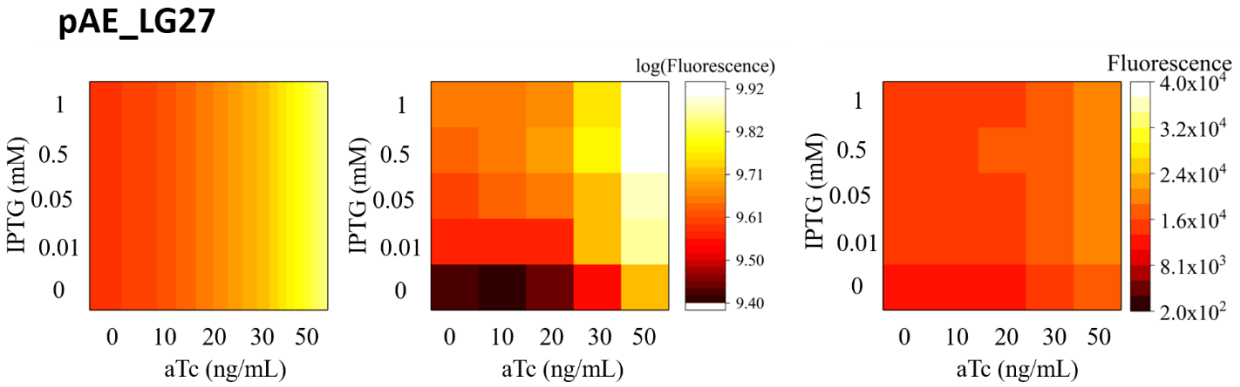
	value	+/-
RNAPbind2	1.58E-03	4.57E-04
a1	1.83	0.59
a2	0.41	0.03

AIC	-84.30
BIC	-80.64
RMSE	0.18
R^2	0.98

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	707	1001	696	941	3978
0.5	2697	768	3126	1082	1288
0.02	1774	2070	2139	2837	3134
0.01	4120	2780	2773	3262	2982
0	28	93	188	542	1022
aTc	0	10	20	30	50

Supplementary Figure S29:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for OR gate pAE_LG26.



	value	+/-
RNAPbind2	4.94	3.73E+06
a1	0.04	0.14
a2	0.37	18.36
m	1.34	2.65
n	2	1.10E+08	

AIC	-112.75
BIC	-106.65
R^2	0.61
RMSE	0.10

IPTG	Flow Cytometry Std. Deviation (n>=4)				
1	2114	1584	1191	1228	2022
0.5	1535	1541	1872	1785	2288
0.02	1394	1448	1617	1409	1628
0.01	1355	1131	1463	1771	1565
0	1502	823	1006	1202	1148
aTc	0	10	20	30	50

Supplementary Figure S30:

Heat maps of (from left to right) fitted log-transformed, log-transformed, and raw GFP expression data are reported, along with fitted parameter values, goodness of fit statistics, and standard deviations from biological replicates for OR gate pAE_LG27.

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