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# Modular, Multi-Input Transcriptional Logic Gating with Orthogonal LacI/GalR Family Chimeras

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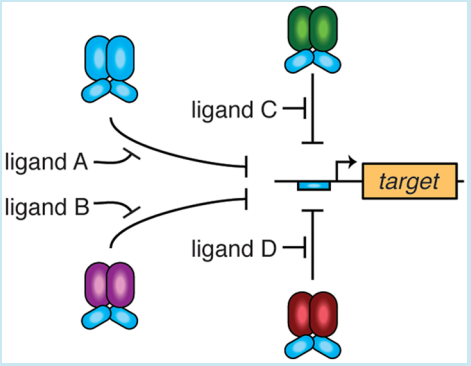
Supporting Information

**ABSTRACT:** In prokaryotes, the construction of synthetic, multi-input promoters is constrained by the number of transcription factors that can simultaneously regulate a single promoter. This fundamental engineering constraint is an obstacle to synthetic biologists because it limits the computational capacity of engineered gene circuits. Here, we demonstrate that complex multi-input transcriptional logic gating can be achieved through the use of ligand-inducible chimeric transcription factors assembled from the LacI/GalR family. These modular chimeras each contain a ligand-binding domain and a DNA-binding domain, both of which are chosen from a library of possibilities. When two or more chimeras have the same DNA-binding domain, they independently and simultaneously regulate any promoter containing the appropriate operator site. In this manner, simple transcriptional AND gating is possible through the combination of two chimeras, and multiple-input AND gating is possible with the simultaneous use of three or even four chimeras. Furthermore, we demonstrate that orthogonal DNA-binding domains and their cognate operators allow the coexpression of multiple, orthogonal AND gates. Altogether, this work provides synthetic biologists with novel, ligand-inducible logic gates and greatly expands the possibilities for engineering complex synthetic gene circuits.




Transcriptional logic gating is the genetic equivalent of Boolean logic gating that is typically found in electronic circuits.[1] In essence, transcriptional logic gating is the simultaneous regulation of a promoter by two or more transcription factors. If the transcription factors are themselves controlled by inputs such as inducible promoters or ligand binding, the result is a logic gate that regulates the expression of a target gene based upon the presence or absence of the inputs. Thus, logic gates can be used by synthetic biologists to engineer complex genetic programs that elicit desired phenotypic responses within host cells.[2−5]

To date, intense efforts have been made to generate more effective and modular transcriptional logic gates for use in synthetic biology. These efforts have generally fallen within two categories: (1) the creation of hybrid promoters that respond to different classes of known transcription factors, and (2) the genomic mining of existing parts from various organisms for use within a particular host. Hybrid promoters have long been widely used. For instance, the Lac/Ara hybrid promoter created by Lutz and Bujard[6] has been used in a number of synthetic gene circuits, such as the dual-feedback synthetic gene oscillator.[7] In addition, hybrid promoters have been engineered to create different types of logic gates[8−11] and layered to generate complex circuits.[12] To expand the genetic toolbox even further, researchers have also begun to mine the genomes of prokaryotes for new components.[13] For instance, Stanton et al. recently mined prokaryotic genomes for analogues of the tetracycline repressor.[14] Another method for generating transcriptional logic gates is to repurpose existing transcription factors via protein engineering. For instance, Shis and Bennett recently used split versions of T7 RNA polymerase to create a library of transcriptional logic gates that strongly drive downstream expression.[15] Here, we take a protein engineering approach to utilize and expand upon a collection of engineered LacI/GalR family chimeric transcription factors[16,17] to create novel transcriptional logic gates, as outlined in Figure 1.

The lactose repressor, LacI, regulates the *lac* operon, which is responsible for the uptake and metabolism of lactose.[18] LacI binds the *lac* promoter at the operator site, $O^1$, to prevent transcription of downstream genes by RNA polymerase. In the presence of the ligand allolactose, or of its nonhydrolizable analogue isopropyl $\beta$-D-1-thiogalactopyranoside (IPTG), LacI loses its high affinity for the operator, which allows transcription to proceed.

LacI is part of a larger LacI/GalR family of transcriptional repressors that regulate sugar metabolism in *E. coli*.[19] Most members of the family respond to their own ligand inducers in much the same way LacI responds to IPTG. Meinhardt et al. recently created a library of chimeric repressors by replacing the ligand binding domain (LBD) of LacI with the LBDs of homologous LacI/GalR family members. This created a set of

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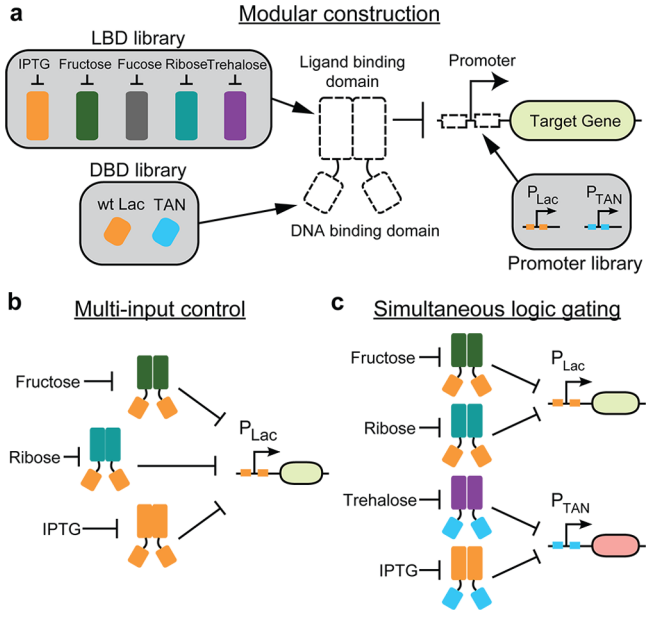


**Figure 1.** Schematic of transcriptional logic gates using LacI/GalR repressors. (a) Each chimera consists of a LBD and a DBD. The set of five LBDs each responds to a different sugar.[16,17] The two DBDs regulate two orthogonal promoters.[20] (b) Combinatorial AND transcriptional logic is facilitated by the simultaneous coexpression of multiple chimeric transcriptional repressors. Since each chimera regulates the same promoter, the ligand for each chimera must be present for downstream transcription to occur. (c) Coexpression of multiple chimeric repressors with a mix of the two DBDs creates functionally orthogonal transcriptional AND gates that regulate the expression of two different genes.

LacI-like transcriptional repressors that respond to the natural inducer of the LBD while regulating transcription of the *lac* promoter.[16,17]

Here, we demonstrate that chimeric LacI/GalR family transcription factors can be used to create modular logic gates in *E. coli* that display tightly regulated ON and OFF states. We use ligand-binding domains from five different members of the LacI/GalR family, each with a different sugar ligand inducer. We show that these five chimeras can be used to create traditional two-input AND gates and that they can also be used *en masse* to enable three- and four-input AND gating. Importantly, the multi-input AND gates we generate are much simpler than existing technologies that have similar functionality.[2] This is because ligand control works directly at the level of each transcription factor, eliminating the need for ligand inducible promoters to control each input. To further increase the modularity of the transcription factors, we also engineered a previously described DNA binding mutation into the DNA binding domain (DBD) of each chimera.[20] These orthogonal DBDs allow the chimeras to regulate promoters that are orthogonal to the *lac* promoter and hence enable multiple types of AND gates to be used simultaneously with minimal cross-talk.

## RESULTS

We first constructed a set of chimeric repressors from the LacI/GalR family transcriptional repressors previously described by Meinhardt et al.,[16,17] specifically the chimeras LLhR_Q60A, LLhS_Q54A_E230K, LLhF_Q60S, and LLhT_V52A, hereafter referred to as RbsR-L, GalS-L, FruR-L, and TreR-L, respectively. The chimeras each contain the DNA binding domain of LacI and the ligand binding domain of repressors that respond to ribose (RbsR-L), fucose (GalS-L), a fructose metabolite (FruR-L), and a trehalose metabolite (TreR-L). When a dimeric version of wild-type (wt) LacI (here called LacI-L for consistency) is included,[21] these proteins form a set of five repressors that derepress in the presence of five different sugars (Figure 1). In addition, we modified the DBD of these transcriptional regulators to include the point mutations Y17T, Q18A, and R22N (the "TAN" mutation), previously described by Daber et al.[20] to create the functionally orthogonal transcriptional repressors, RbsR-T, GalS-T, FruR-T, TreR-T, and LacI-T (Supporting Information Table S1).

We tested the ability of each chimeric repressor to regulate the expression of a gene encoding green fluorescent protein (*gfp*) driven by either the Lac promoter, $P_{Lac}$, or the orthogonal TAN promoter, $P_{TAN}$ (Supporting Information Figure S1). The expression of *gfp* was repressed by each chimera and induced in the presence of the appropriate sugar for both sets of DBDs. We also confirmed the orthogonality of the two DBDs by testing for regulated GFP fluorescence from either $P_{Lac}$ or $P_{TAN}$ in the presence of the opposite repressor, LacI-T or LacI-L, respectively (Supporting Information Figure S2). As expected, when the chimera with the wrong DBD was used, fluorescence was the same in the absence or presence of IPTG, indicating no repression occurred.

While the use of individual LacI/GalR chimeras allows flexibility in ligand choice, coexpressing multiple chimeric repressors enables combinatorial transcriptional AND logic. For instance, coexpressing two chimeras with the same DBD should create a transcriptional AND gate for which the inputs are sugars specified by each chimera's LBD. Since both chimeras repress the same promoter, expression of the target gene should occur if and only if both sugars are present. To test this hypothesis, we measured the regulation of *gfp* driven by the $P_{Lac}$ promoter in the presence of LacI-L and each of the four other chimeras (Figure 2). For three combinations, GFP fluorescence was observed if and only if inducing amounts of both IPTG and the relevant sugar were present in the media

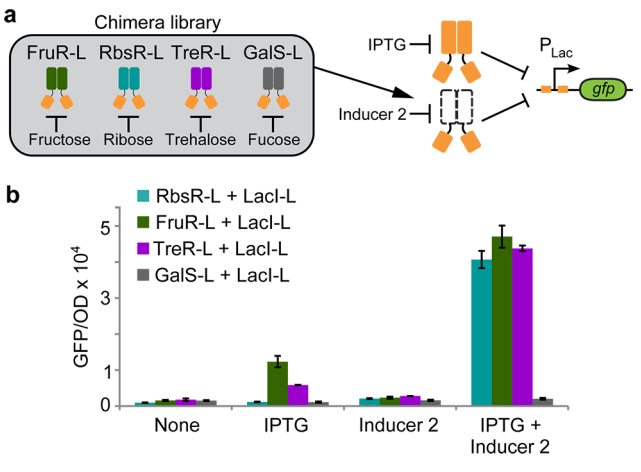


**Figure 2.** Pairs of engineered LacI/GalR repressors with the same DBD create AND transcriptional logic. (a) Each LacI/GalR chimeric repressor was coexpressed with LacI-L and the induction of *gfp* driven by $P_{Lac}$ was monitored. (b) GFP fluorescence in response to all combinations of the presence or absence of 10 mM IPTG and 10 mM of the respective LBD's inducer. Data points and error bars reflect the average and standard deviation of three experimental replicates.


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(Figure 2b). However, the combination of LacI-L and GalS-L showed no induction, even in the presence of IPTG and fucose. Subsequent investigation revealed that IPTG inhibits the ability of fucose to induce expression of a GalS-L regulated system (Supporting Information Figures S3 and S4), similar to what happens for GalR in the presence of IPTG.[16,22] We also tested every pairwise combination of sugars with each chimera and found that the IPTG inhibition of GalS-L was the only instance of competitive inhibition in this set of chimeras (Supporting Information Figure S3).

We then expanded this study to include all pairwise combinations of the chimeras with either the wt *lac* or TAN DBD. We expressed pairwise combinations of each chimera and tested for the induction of GFP fluorescence from $P_{Lac}$ in the presence or absence of 10 mM of each appropriate inducer (Figure 3a). Except for the pairings of GalS-L and FruR-L in addition to GalS-L and LacI-L, all pairwise combinations of

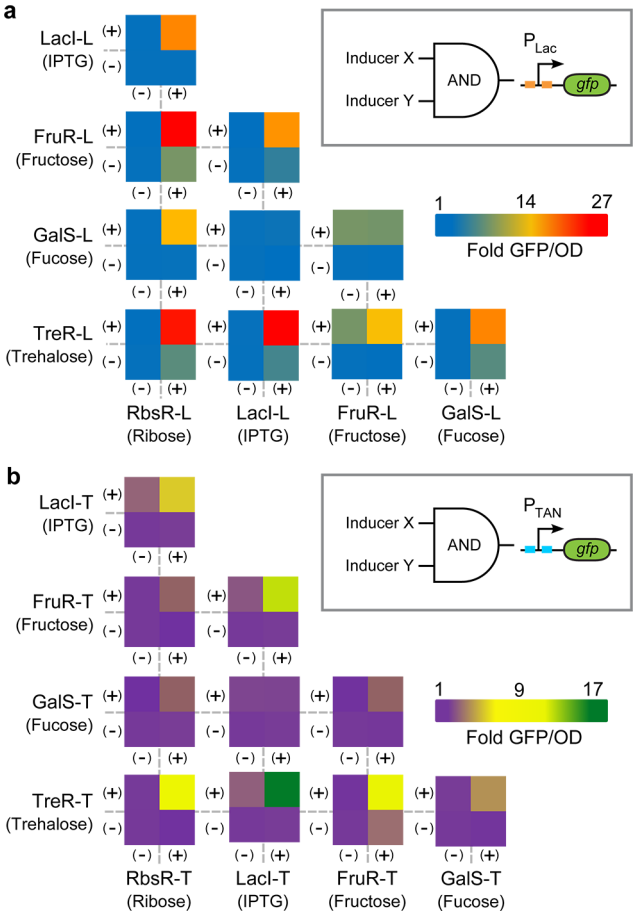


**Figure 3.** Pairwise combinations of repressors lead to AND transcriptional logic. (a) All repressors contained the wt *lac* DBD in this experiment. Pairwise combinations of transcription repressors were coexpressed, and the induction of GFP fluorescence from $P_{Lac}$ was monitored in the presence and absence of 10 mM of each transcription factor's inducer. (b) All repressors contained the TAN DBD in this experiment. Pairwise combinations of transcriptional repressors were coexpressed and the induction of GFP fluorescence from $P_{TAN}$ was monitored in the presence or absence of 10 mM of each inducer for each transcription factor. "Fold GFP/OD" is the observed fluorescence/OD for a given inducer condition normalized to no inducer. Each data point represents the average of three experimental replicates. Error bars can be found in Supporting Information Figures S5 and S6.

transcription factors facilitated AND transcriptional logic: GFP fluorescence was observed if and only if inducing amounts of inducers for both repressors were present.

We next tested for AND transcriptional logic at the promoter $P_{TAN}$ when pairs of chimeras with the TAN DBD were coexpressed (Figure 3b). Similar to the wt DBD, many of the combinations facilitated AND transcriptional logic at $P_{TAN}$, and maximal GFP fluorescence was observed only in the presence of both inducers. However, in most instances the overall induction level from $P_{TAN}$ was much lower than that of $P_{Lac}$ and generally consistent with induction levels of each chimera when used alone (Supporting Information Figure S1).

Since expressing pairs of repressors creates AND transcriptional logic, we hypothesized that coexpression of three or more repressors would create a multi-input transcriptional AND gate. In that case, induction of gene expression would be observed if and only if inducing ligands for all transcription factor were present (Figure 4). Therefore, we first created a three-input gate (IPTG, ribose, and fructose) by controlling the promoter $P_{Lac}$ with the LacI-L, FruR-L, and RbsR-L repressors. We assayed for GFP fluorescence with all combinations of 10 mM of IPTG, fructose, and ribose. As predicted, induced GFP fluorescence was observed if and only if all three ligands were present, while background was observed otherwise (Figure 4a). We next constructed a four input AND gate with the inputs of IPTG, fructose, ribose, and threhalose by coexpressing the repressors, LacI-L, FruR-L, RbsR-L, and TreR-L. Again, induced GFP fluorescence was seen only in the presence of the four inducers IPTG, fructose, ribose, and trehalose (Figure 4b).

Finally, we hypothesized that the coexpression of chimeric repressors with a mixture of the wt *lac* and TAN DBDs would allow simultaneous logic gating at different gene outputs. Therefore, we coexpressed repressors possessing either the wt *lac* or TAN DBD and assayed for gene expression from promoters regulated by either the wt *lac* or the TAN DBD. We first tested a wt *lac* transcriptional AND gate working in conjunction with $P_{TAN}$ regulated by a single repressor with the TAN DBD. We coexpressed the chimeras FruR-L and RbsR-L and LacI-T and tested for inducible fluorescence from the wt *lac* DBD regulated promoter $P_{Lac}$ (driving *mCherry*) and from TAN DBD regulated promoter $P_{TAN}$ (driving *gfp*) (Figure 5a). As expected, we observed induced mCherry fluorescence only in the presence of fructose and ribose, while GFP fluorescence was seen only in the presence of IPTG (Figure 5b).

We extended this system to contain two functionally orthogonal transcriptional AND gates, one at the promoter $P_{Lac}$ (driving *mCherry*) and the other at $P_{TAN}$ (driving *gfp*) by coexpressing the chimeras FruR-L, RbsR-L, LacI-T, and TreR-T (Figure 5c). We tested for GFP and mCherry fluorescence in response to all combinations of 10 mM IPTG, fructose, ribose, and trehalose. We observed induced mCherry fluorescence only when ribose and fructose were present and induced GFP fluorescence only when IPTG and trehalose were present (Figure 5d). This demonstrated that the two transcriptional logic gating systems could be used simultaneously with minimal cross-talk.

## DISCUSSION

Here, we have shown that the construction of modular, multi-input transcriptional logic gates is possible through the use of ligand-inducible transcription factor chimeras. These chimeras make use of two main properties. First, by using transcription


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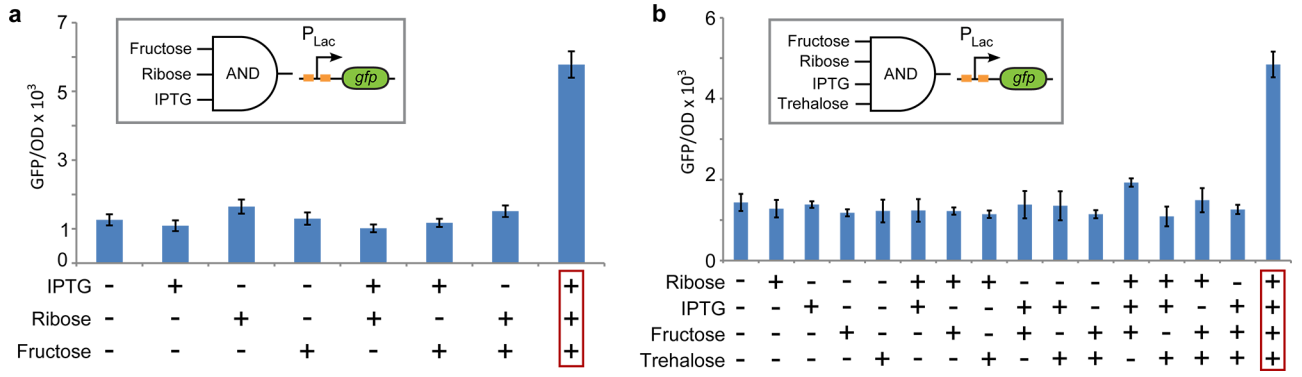


**Figure 4.** Multi-input transcriptional AND gating. Three (a) and four (b) chimeras were coexpressed to create multi-input AND gates. Each chimera contained a different LBD and the wt *lac* DBD. *gfp* was driven by $P_{Lac}$. (a) For three-input transcriptional AND logic, we coexpressed the repressors LacI-L, FruR-L, and RbsR-L and assayed for GFP fluorescence in the presence and absence of all combinations of 10 mM IPTG, fructose, and ribose. (b) For four-input transcriptional AND logic, we coexpressed the repressors LacI-L, TreR-L, RbsR-L, and FruR-L and assayed for inducible GFP fluorescence in the presence or absence of 10 mM IPTG, fructose, ribose, and trehalose. Both plots show the mean GFP/OD and error bars reflect the standard deviation of three experimental replicates.

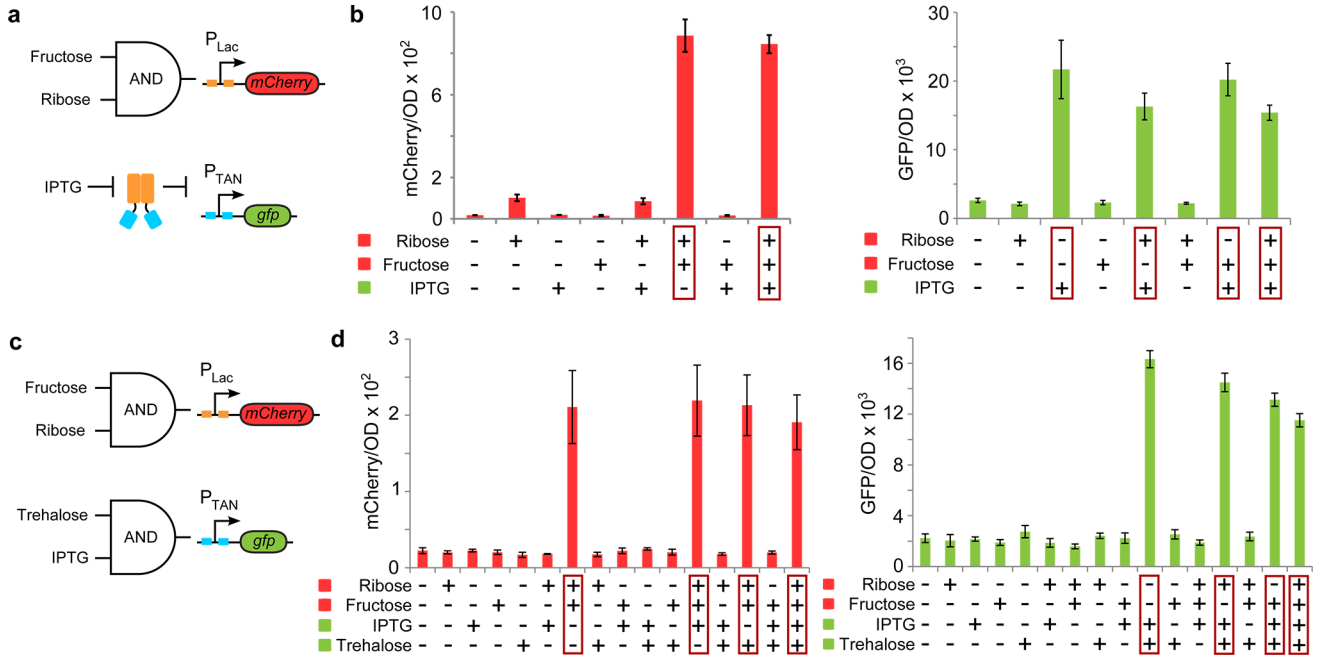


**Figure 5.** Coexpression of repressors with either the wt *lac* DBD or the TAN DBD for transcriptional logic at two orthogonal promoters. (a) The chimeric repressors FruR-L and RbsR-L regulating $P_{Lac}$, in addition to LacI-T regulating $P_{TAN}$, were coexpressed with a dual reporter plasmid containing $P_{Lac}$ driving *mCherry* expression and $P_{TAN}$ driving *gfp* expression. (b) Induction of GFP and mCherry fluorescence in the presence of absence of 10 mM IPTG, fructose, and/or ribose. (c) Coexpression of the repressors FruR-L and RbsR-L, both regulating $P_{Lac}$, and TreR-T and LacI-T, regulating $P_{TAN}$, behaved as two orthogonal transcriptional AND gates for the dual reporter plasmid. (d) GFP and mCherry fluorescence was assayed in the presence or absence of 10 mM fructose, ribose, trehalose, and/or IPTG. Conditions for which fluorescence was expected are highlighted with red boxes in b and d. Data points and error bars reflect the average and standard deviation of three experimental replicates.

factors from the same structural family, namely the LacI/GalR family, one can swap ligand binding domains from one transcription factor to another.[16,17] This type of domain swapping has been used in other contexts, as well. For instance, Temme et al. were able to engineer orthogonal versions of T7 RNA Polymerase by replacing the specificity loop with a loop from a distant homologue.[5]

The second main property used in this study was the plasticity of the DNA-binding domains. Through mutation, the DNA binding domain of LacI can be altered to recognize an alternate promoter.[20,23] Similar studies have been used to alter the promoter specificity of T7 RNA polymerase.[5,24−26] This type of mutation is particularly powerful, as it allows for the coexpression of similar regulatory proteins that target orthogonal pathways. In addition, because the overall function of the transcription factors remains unchanged, they can be easily implemented within large scale circuits with minimal retuning of regulatory strengths.[27,28]

This study can be further expanded in several ways. First, the LacI/GalR family comprises many more proteins that could be used. Swint-Kruse and co-workers showed that at least nine different ligand binding domains of these family members could be fused to the LacI DNA binding domain to create functional repressors.[16,29,30] However, not all of these chimeras show strong inducibility, and some have significant cross-talk between sugar ligands.[16] This cross-talk could be used as an


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additional layer of logic. For instance, the GalS-L chimera used in this study is competitively inhibited by IPTG. This means that any logic gate in which GalS-L is used will also contain NOT (IPTG) logic (i.e., will not be active in the presence of IPTG). Further engineering might also eliminate undesirable cross-talk. For example, a point mutation within the linker domain of FruR-L improves the responsiveness of the chimera to ligand.[17] Second, other families of transcription factors might be used in a similar way. For example, the growing body of knowledge on the AraC/XylS family of transcriptional activators suggests that a similar library of ligand-inducible transcription factors may be possible.[31−33]

Of course, logic gating in protein regulation is not limited to the transcriptional level. Recent studies have used other modalities of translation and transcriptional regulation such as amber stop codon suppression[34] and DNA recombination to engineer conditional gene regulation.[4,35] Nor is logic gating exclusively limited to prokaryotes, as recent advances in eukaryotic gene regulation demonstrate.[36−38] Taken together, these new tools provide powerful components with which to program gene regulation at transcriptional[39] and post-transcriptional levels.[3]

## METHODS

**Rationale for Choosing Chimera Mutants.** The particular chimeras were selected from a set of more than 1000 variants for two main reasons: (1) they have large regulatory ranges as a function of ligand concentration,[17] and (2) their minimal and maximal repression levels are well-matched among the set. In some cases, we found that fine-tuning the strength of the promoters driving each gene gave better results. In particular, some chimeras worked better when their genes were driven by the $P_I$ promoter, while others worked better with $P_{Iq}$.[40]

Note that chimeras with the FruR and TreR LBD are induced by fructose-1-phosphate and trehalose-6-phosphate, respectively. However, we used D-fructose and D-trehalose, which are each phosphorylated upon cellular import.

**Assay for Fluorescent Protein Production.** Single colonies were inoculated in to 2 mL selective 0.04% glycerol modified MOPS minimal media and grown overnight (M2101, Teknova).[16] 0.8% glycerol modified MOPS minimal media was then inoculated with the overnight culture at 1% v/v. Inducer was added and each culture was then aliquoted in triplicate into a 96 well U-bottom plate (BD Falcon 35117). Each plate was incubated at 37 °C and shaken at 800 rpm in a microplate shaker (VWR, 12620-926) for 3 h. Plates were then assayed for growth (OD, 600), GFP fluorescence (ex, 488 nm; em, 510 nm), and/or mCherry fluorescence (ex, 587 nm; em, 610 nm) (Tecan M1000, Tecan). All fluorescence values were normalized to growth (OD, 600). Each data point and error bar represents the average and standard deviation of three experimental replicates, respectively.

Modified MOPS minimal media was used exclusively in this study to ensure consistent induction of gene expression by each sugar sensitive LacI/GalR chimeric repressor. Inconsistencies in the preparation of LB media between research groups lead to inconsistent induction of gene expression in our experiments.

All experiments were performed in the cell strain JS006, which is the *E. coli* strain MG1655 that has *lacI* and *araC* knocked out.[7] In experiments testing the induction of gene expression in the presence of more than one chimera, excess amounts (10 mM) of inducer was used. This was to detect cross-talk between LBDs. No deleterious effects on cell growth were observed due to the high levels of inducer.

When possible, expression of each transcriptional repressor was controlled using the strong constitutive promoter $P_{Iq}$.[40] For LacI-L, LacI-T, TreR-L, and TreR-T, we used $P_I$. This increased the range of induction for LacR-R and LacI-T (Supporting Information Figure S1). For TreR-L and TreR-T, this allowed detectable induction that was absent when $P_{Iq}$ was used.

Plasmids maps and genotypes used in this study are provided in Supporting Information (Figure S7 and Table S2).

**AND Transcriptional Logic with LacI/GalR Chimeras.** To confirm the ability of each transcriptional repressor to regulate gene expression with the wt *lac* DBD, we cotransformed the plasmids pZS1[FruR-L], pZS1[RbsR-L], pZS1[GalS-L], pZS1[TreR-L], and pZS1[LacI-L] with pZA2-[$P_{Lac}$:GFP] into JS006 cells. To test the abilities of repressor with the TAN DBD to regulate gene expression, we cotransformed the plasmids pZS1[FruR-T], pZS1[RbsR-T], pZS1[GalS-T], pZS1[TreR-T], and pZS1[LacI-T] with the GFP expression plasmid pZA2[$P_{TAN}$:GFP] into JS006 cells. Induction of GFP fluorescence from the GFP reporter plasmids was then assayed as a function of each chimera LBD's inducer.

To test the functional orthogonality of the wt *lac* and TAN DBDs, we cotransformed the plasmid pZS1[Iq:LacI-L] with pZA2[$P_{TAN}$:GFP] and pZS1[Iq:LacI-T] with pZA2[$P_{Lac}$:GFP] into JS006 cells. In these expression systems, the repressor was driven by the "strong" promoter $P_{Iq}$.[40] GFP fluorescence was assayed with or without 10 mM IPTG.

To coexpress the chimeras FruR-L, RbsR-L, TreR-L, and GalS-L with LacI-L, the expression plasmids pZS1[RbsR-L, LacI-L], pZS1[FruR-L, LacI-L], pZS1[GalS-L, LacI-L], and pZS1[TreR-L, LacI-L] were each cotransformed with the GFP expression plasmid pZA2[$P_{Lac}$:GFP] into the strain JS006. GFP fluorescence was assayed in the presence or absence of 10 mM IPTG and or the inducer of the chimera's LBD.

To test the regulation of GFP fluorescence when GalS-L was in the presence of IPTG, the plasmids pZS1[GalS-L] was cotransformed with pZA2[$P_{Lac}$:GFP] into JS006 cells. Induced GFP fluorescence was then assayed in the presence of different fucose concentrations as a function of increasing IPTG concentration.

To test all pairwise combinations of the chimeras with the lac DBD, the plasmids pZS1[FruR-L, RbsR-L], pZS1[FruR-L, TreR-L], pZS1[GalS-L, RbsR-L], pZS1[GalS-L, FruR-L], pZS1[GalS-L, TreR-L], and pZS1[RbsR-L, TreR-L] were cotransformed with pZA2[$P_{Lac}$:GFP] into the strain JS006. GFP fluorescence was assayed in the presence or absence of 10 mM of the inducer for each transcriptional regulator.

Similarly, to test AND transcriptional logic at the promoter $P_{TAN}$:GFP, the plasmids pZS1[RbsR-T, LacI-T], pZS1[FruR-T, LacI-T], pZS1[GalS-T, LacI-T], pZS1[TreR-T, LacI-T], pZS1-[FruR-T, RbsR-T], pZS1[FruR-T, TreR-T], pZS1[GalS-T, RbsR-T], pZS1[GalS-T, FruR-T], pZS1[GalS-T, TreR-T], and pZS1[RbsR-T, TreR-T] were each cotransformed into JS006 cells with the reporter plasmid $P_{TAN}$:GFP. GFP fluorescence was measured in the presence and absence of all combinations of 10 mM of each inducer for each transcription factor.

**Multi-Input AND Transcriptional Logic with LacI/GalR Chimeras.** To test the multi-input AND transcriptional logic gates, we coexpressed three or four chimeras to facilitate three and four input AND transcriptional logic, respectively. For a three input AND gate at the promoter $P_{Lac}$, the plasmids

pZS1[LacI-L] and pzS3[I:FruR-L,I:RbsR-L] were cotransformed with the reporter plasmid pZA2[$P_{Lac}$:GFP] into JS006 cells. To create a four-input transcriptional AND gate, the plasmids pZS1[TreR-L,LacI-L] and pZM3[I:FruR-L,RbsR-L] were transformed with pZA2[$P_{Lac}$:GFP] into the strain JS006. For both the three and four input transcriptional AND gates, induction of GFP fluorescence was assayed in the presence and absence of all combinations of 10 mM inducer of reach chimera.

**Multi-ORF and AND Transcriptional Logic with LacI/GalR Chimeras.** To test the simultaneous regulation of orthogonal ORFs with both the wt *lac* and TAN DBDs, we expressed chimeras with either DBD with the dual reporter plasmid containing wt *lac* DBD regulatable promoter $P_{Lac}$ and the TAN DBD regulatable promoter $P_{TAN}$ driving expression of mCherry and GFP, respectively. To test a transcriptional AND gate working in conjunction with a single regulator with the TAN DBD, we cotransformed the plasmids pZS1[LacI-T] and pZM3[FruR-L,RbsR-L] with the reporter plasmid pZA2-[$P_{Lac}$:mCherry,$P_{TAN}$:GFP]. Induction of GFP and mCherry fluorescence was assayed in the presence or absence of all combinations of 10 mM of each inducer for each transcriptional regulator. Similarly, to test the preparation of two orthogonal transcriptional AND gates, we cotransformed the plasmids pZS1[TreR-T, LacI-T] and pZM3[I:FruR-L,I:RbsR-L] with the dual reporter plasmid pZA2[$P_{Lac}$:mCherry,$P_{TAN}$:GFP] and then assayed the induction of GFP and mCherry fluorescence was assayed in the presence or absence of all combinations of 10 mM of each inducer for each chimeric repressor.

## ■ ASSOCIATED CONTENT

### Supporting Information

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### Notes

The authors declare no competing financial interest.

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