Engineering the Ultrasensitive Transcription Factors by Fusing a Modular Oligomerization Domain

ACS Synthetic Biology

Letter

Engineering the ultrasensitive transcription factors by fusing a modular oligomerization domain

Junran Hou, Weiqian Zeng, Yeqing ZONG, Chensi Miao, Baojun Wang, and Chunbo Lou

ACS Synth. Biol., **Just Accepted Manuscript** • DOI: 10.1021/acssynbio.7b00414 • Publication Date (Web): 07 May 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Engineering the ultrasensitive transcription factors by fusing a modular oligomerization domain

Junran Hou[1,3†], Weiqian Zeng[2,†], Yeqing Zong[1,3], Zehua Chen[1,3], Chensi Miao[1,4], Baojun Wang[5,6], Chunbo Lou[1,2,3,*]

[1]CAS Key Laboratory of Microbial Physiological and Metabolic Engineering and Institute of Microbiology, State Key Laboratory of Microbial Resources, Institute of Microbiology Chinese Academy of Sciences, Beijing, 100101, China

[2]Institute of Molecular Precision Medicine, The Xiangya Hospital, Central South University, Changsha, Hunan, 410008, P. R. China

[3]College of Life Science, University of Chinese Academy of Science, Beijing, 100149, China

[4]College of Life Sciences, University of Science and Technology of China, Hefei 230027, China

[5]School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3FF, UK

[6]Centre for Synthetic and System Biology, University of Edinburgh, Edinburgh EH9 3FF, UK

[*]Corresponding author. Tel: +86-10-6480 6105; E-mail: louchunbo@im.ac.cn

[†]These authors contributed equally to this work.

Abstract

The dimerization and high-order oligomerization of transcription factors has endowed them with cooperative regulatory capabilities that play important roles in many cellular functions. However, such advanced regulatory capabilities have not been fully exploited in synthetic biology and genetic engineering. Here, we engineered a C-terminally fused oligomerization domain to improve the cooperativity of transcription factors. First, we found that two of three designed oligomerization domains significantly increased the cooperativity and ultrasensitivity of a transcription factor for the regulated promoter. Then, seven additional transcription factors were used to assess the modularity of the oligomerization domains, and their ultrasensitivity was generally improved, as assessed by their Hill coefficients. Moreover, we also demonstrated that the allosteric capability of the ligand-responsive domain remained intact when fusing with the designed oligomerization domain. As an example application, we showed that the engineered ultrasensitive transcription factor could be used to significantly improve the performance of a "stripe-forming" gene circuit. We envision that the oligomerization modules engineered in this study could act as a powerful tool to rapidly tune the underlying response profiles of synthetic gene circuits and metabolic pathway controllers.

Keywords: transcription factor; ultrasensitivity; high-order oligomerization domain; genetic circuit; stripe-forming function; Hill coefficient.

Transcription factors are central cellular regulators in natural and synthetic biological systems [1-3]. A typical transcription factor is generally composed of several functional domains, including a DNA-binding domain to recognize the specific DNA sequence, an allosteric domain to respond to cellular signals, and an oligomerization domain to increase the regulatory cooperativity [4]. Numerous DNA-binding domains have been engineered to recognize new sequences [5, 6], and some allosteric domains have been mutated to specifically respond to new ligands [7, 8]. For example, the arabinose-responsive AraC domain has been engineered to recognize an important metabolic intermediate, mevalonate [9]. However, thus far, oligomerization domains have not been fully exploited in engineered biological systems.

The oligomerization of transcription factors typically leads to DNA looping on the regulated promoters, which is regarded as an important transcriptional regulatory mechanism in both prokaryotic and eukaryotic cells [10, 11]. Several transcription factors, such as LacI, LysR, CI, and P53, can form tetramers or higher-order oligomers when binding to their multi-adjacent operators [12-15], and their native forms have been used to increase the ultrasensitivity and reduce the basal expression in synthetic gene systems [16, 17]. However, most transcription factors lack such high-order oligomerization domains, and thus are incapable of generating higher cooperativity in their regulatory systems, resulting in imperfect performances in the engineered systems. For example, FapR as a Malonyl-CoA sensor only generate less than 5-fold dynamic range when dynamically controlling the conversion from Malonyl-CoA to biofuels, no matter how to vary the combination of operators and promoter, possibly due that the native FapR is incapable of cooperatively binding these operators. [18, 19].

We have developed a modular high-order oligomerization domain, which generally and effectively improved the cooperativity for transcription factors. To validate the generalizability of the domain, we tested the domain on a diverse set of transcription factors, and the results showed their cooperativity were dramatically increased. Meanwhile, the modularity of the oligomerization domain was supported by the intact allosteric capability of the fused ligand-responsive sensors. To demostrate the application of the modular oligomerization domain, a chimeric LmrA repressor was integrated into an incoherent feedforward gene circuit, which led to a significantly improved bandpass filtering function of the circuit.

Results

The design of synthetic high-order oligomerization domains and cognate regulated promoters

Inspired by the natural DNA looping regulation of the *lac* operon in *Escherichia coli* [15], we first selected the oligomerization domain of the LacI repressor, and fused it to the C-terminus of a CymR repressor that was dimeric but lacked high-oligomerization capability (Figure 1A). Two operators were placed separately on the upstream and downstream of a tested promoter (named O_U and O_D,

respectively) to facilitate the binding of each CymR dimer to each CymO operator, and a reporter gene (*sfgfp*) was used to measure the regulated promoter activity (see the Methods section and supplementary Figure S1 for details). We expected that the two engineered CymR dimers would cooperatively bind to the two CymO operators, and improve their repression capability and ultrasensitivity for the regulated promoter activity (Figure 1B). However, we found that the tetramer domain of LacI (named $LacI_{oligomer}$) only slightly increased the repression capability in the chimeric CymR-$LacI_{oligomer}$ repressor, possibly due to the 20-residue $LacI_{oligomer}$ domain being too small to form a stable and functional structure in the chimeric repressor (gray line in Figure 1B). Next, we chose two larger oligomeric domains from the CI and CI434 repressors, *i.e.* 93-236 amino acid (aa) of CI repressor and 70-210aa of CI434 repressor (named $CI_{oligomer}$ and $CI434_{oligomer}$, respectively) [20, 21], to evaluate their effect on the chimeric CymR repressor. We found that both the $CI_{oligomer}$ and $CI434_{oligomer}$ domains successfully improved the repression ability of the chimeric CymR (Figure 1B). A common feature of the two domains was they were folded into independent domains in their native proteins[13, 22], indicating that the independent folding of the oligomerization domain (named "oligomeric domain") was important for modularly maintaining their cooperative capability in the chimeric transcription factors.

Next, we added more flexible linkers to further improve the flexibility and modularity of the oligomeric domains. The GGGGS and $(GGGGS)_2$ peptides, as flexible linkers, were inserted between the oligomeric domains and the CymR repressor. In comparison with the naked $CI434_{oligomer}$ domain, the two additional linkers slightly increased the repression ability of the chimeric CymR (Figure 1C). In the following study, we chose the longest linker $(GGGGS)_2$ as our designed linker to bridge the oligomeric domains and the transcription factors.

The oligomeric domains increased the ultrasensitivity of all tested transcriptional repressors.

To quantify the ultrasensitivity of transcriptional regulation, Hill coefficients (Equation 1) were used to characterize the slopes of the input-output response curves of the repressor and regulated promoter systems. The transcriptional level of the repressor as input and the activity of the regulated promoter as output were measured independently by a reporter gene as described in our previous report[23], briefly, the input promoter activity and cognate output promoter activity were independently detected by a same reporter gene (*sfgfp*), and titrated with the IPTG inducers (Figure S1). We first compared the wild-type and engineered LmrA to evaluate the contribution of oligomeric domains on their ultrasensitivity. For convenience, the engineered oligomeric LmrA (LmrA-$CI434_{oligomer}$) was named as $LmrA^*$ (the same naming rule is applied to other engineered repressors; e.g., $CymR^*$ for the chimeric CymR). With the oligomeric domain, the Hill coefficient of the $LmrA^*$ repressor was increased from 1.9 to 3.3 on the same dual operator (O_U-O_D), when comparing with the wild type LmrA (Figure 2A, red line vs. blue line). Without the oligomeric domain in the repressor, the additional upstream

operator did not significantly improve the Hill coefficient in the dual operator (O_U-O_D) (Figure 2A, blue line vs. black line). These results supported that the wild type LmrA repressor inherently lacked tetra- and higher-order oligomerization capabilities. Similarly, we found that without the additional operator (O_U), the chimeric LmrA* alone did not improve the Hill coefficient (Figure 2B). Based on the previous study, we also demonstrated that two extra operators (O_{dis}) located on the 200 base pair (bp) upstream of the dual operator (O_U-O_D) further increased the Hill coefficient from 3.3 to 4.4 by the DNA looping regulation (Figure 2C), indicating that an octamer might have formed and improved the ultrasensitivity of the chimeric LmrA* for its regulated operators. In order to further investigate the length effect of DNA looping on the Hill coefficient, two lengths (500 bp and 700 bp) were inserted between the extra operators (O_{dis}) and the dual operators (O_U-O_D), and the results showed that their Hill coefficients were similar to the 200 bp length (Figure S2).

To estimate the generalization of the cooperative capability, we selected seven other diverse repressors, and fused them with the CI434$_{oligomer}$ oligomeric domain. Among the selected repressors, three (Cro, MazE, and YefM) were composed of a single domain responsible for both DNA-binding and weak dimerization [24-26], while AmtR contained two separate domains responsible for DNA-binding and dimerization functions [27]. Three ligand-responsive repressors (PhlF, CymR, and TrpR) contained one DNA-binding domain and one dimerization domain to sense specific metabolites [28-31]. As shown in Figure 2D, the oligomeric domain dramatically improved the repression capability and Hill coefficients of all the tested transcription factors (Figure 2D). The only unusual case was the AmtR* repressor, for which both the Hill coefficients and repression capability were slightly decreased when comparing the chimeric repressor to the wild type AmtR. However, the Hill coefficients and repression capability of the AmtR* repressor were indeed increased when comparing the dual (O_U-O_D) with single (O_D) operators (i.e., shown with the subtitle "*AmtR*(O_D/O_U-O_D)*" in Figure 2D). On the contrary, for the PhlF and Cro repressors, the dimerization affinities were enhanced by the same oligomeric domain, resulting in the increased repression of the chimeric PhlF* and Cro* in the single-operator systems (Figure S3); meanwhile, their tetramerization in the dual operators further improved their repression capability. Taken together, we concluded that the synthetic oligomeric domain increased the tetramerization capability of the transcription factors, but, in a few cases, reduced or enhanced the intrinsic dimerization affinity of the dimeric transcription factors.

The oligomeric domains did not affect the allosteric capability of the ligand-responsive transcription factors.

We next investigated whether the ligand-responsive transcription factors kept their allosteric function when fusing with an oligomeric domain. Two ligand-responsive repressors (CymR and PhlF), in which cumate and 2,4-diacetylphoroglucinol (DAPG) can respectively bind to the CymR and PhlF

repressor, and result in the decrease of their repression on the regulated promoters, were chosen to measure the effects of additional oligomeric domains on their cooperativity and ultrasensitivity (Figure 3). For the CymR, the leaked expression of the regulated promoter for the oligomeric CymR* was reduced 10-fold when compared with that of the cognate wild type repressor. Meanwhile, the fully induced expressions of the regulated promoters were nearly the same for the oligomeric and wild type CymR repressors (Figure 3A). Similarily, the oligomeric domain in the PhlF slightly reduced the leaked expression, but dramatically increased the Hill coefficient, as indicated by the dose-response curve (from 1.61 to 2.42; Figure 3B). This was possibly due to the low leaked expression of the wild type PhlF not being further reduced. More interestingly, both chimeric CymR* and PhlF* repressors had similar sensitivities as their wild type forms regarding their effective inducer concentrations (Figure 3A and 3B). We hypothesized that the oligomeric domain did not affect the sensing and allosteric capability of the tested transcription factor, but dramatically improved their cooperativity or ultrasensitivity for the regulated promoters.

Ultrasensitive chimeric transcription factors predictably tuned and improved the gene circuit functions.

After determining the success of the designed ultrasensitive transcription factors, we tested the cooperativity and ultrasensitivity, to improve the performance of the synthetic gene circuits. We constructed a state-of-the-art incoherent feedforward loop (IFFL) circuit in which an input (D-1-thiogalactopyranoside; IPTG), via a LacI-repressed Ptac promoter, simultaneously regulated the expression of both the LmrA repressor and an activator (T7 RNAP) as a first layer of the gene circuit. As the second layer, the activator and the LmrA repressor co-regulated the same output promoter (Figure 4A), resulting in a stripe-forming function. Based on the characterized dose-response curves of LmrA* and LmrA, we predicted that the cooperation capability of LmrA* would slightly decrease the repression ability in the low- and intermediate-input regions (Figure 2A), but would dramatically increase the repression ability in the high-input region (Figure 2A). Thus, it would more efficiently convert a gradually increased input gradient into central maximal and peaked "stripe-forming" output gene expression [32]. As shown in Figure 4B, the stripe-forming circuit produced an 8.98-fold peak for the chimeric LmrA* repressor (blue line in Figure 4B), but only up to a 3.3–fold peak for the wild type repressor (red line in Figure 4B). We anticipated that the synthetic oligomeric domain, as a modular system part, could be readily integrated into other genetic circuits to significantly tune and improve the desired functions.

Discussion

In this study, we have designed and demonstrated a modular protein oligomerization system to

efficiently improve the ultrasensitivity of transcription factors. These engineered ultrasensitive transcription factors could improve the digital behaviors of biocomputing circuits, such as the logic-gate circuits. The stochastic nature of transcription factors typically limit the fidelity of the biological signal propagation in a living cell; however, the digital-like characteristics of the engineered transcription factors could result in a steep transition between the OFF and ON states for a basic logic-gate [33]. A steep transition would reduce the biological noise propagation within a layered logic-gate circuit, and thus facilitate building large scale gene circuits [34-36].

The engineered $CI_{oligomer}$ and $CI434_{oligomer}$ domains can be modularly fused to the C-terminus of any transcription factor. However, for some transcriptional activators, if the C-terminus is the DNA-binding domain, it could not be fused to the additional oligomeric domain. In such cases, the N-terminal oligomeric domains could be used from the N-terminal oligomerized transcription factors, such as the MvaT from *Pseudomonas aeruginosa* [37] and the P53 from mammalian cells [12]. For example, the oligomerization capability of the intact MvaT activator was validated by measuring the interaction of two chimeric proteins [37].

When fusing the $CI434_{oligomer}$ domain to transcription factors, we noticed they could enhance or reduce the dimerization of some transcription factors. For example, the dimerization affinity of AmtR was decreased, and the affinities of PhlF and Cro were increased by a same oligomeric domain [38]. The reported crystal structures for AmtR and Cro support the above hypothesis. In an AmtR dimer, the two C-terminal ends are far away from each other, and are separated by a large homodimeric domain. Thus, the dimerization of AmtR might be interfered by the fused oligomeric domain. In contrast, for the Cro dimer, the distance between the two C-terminal ends is small, and its native dimerization capability is very weak. Hence, it is reasonable to speculate that the oligomeric domain could disrupt the dimerization affinity for the AmtR repressor, but may promote the stability for the weakly dimerized Cro protein[39]. To eliminate the potential interference between the higher-order oligomeric domain and the homodimeric domains, we will need to develop more flexible linkers or shorten the distance between the two C-terminal ends.

We expect that the modular oligomeric domain could be used in many other prokaryotic cells and eukaryotic systems. We have introduced the chimeric $PhlF^*$ and $CymR^*$ regulators into *Streptomyces* and a mammalian cell line as advanced genetic switches, and the preliminary data indicated strong cooperativity existing for the above dual operators systems, but more quantitative data need to validate the above speculations. Therefore, we suggest that the modular cooperative components engineered in this study could be powerful tools for synthetic biology, to aid in the design of sophisticated genetic circuits, or temporally-regulated metabolic pathways in a wide variety of engineered hosts.

Materials and methods

Media

Bacterial strains were grown in Luria-Bertani (LB) (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) (Fisher Scientific, Waltham, MA, USA) liquid media or on agar plates (media with 1.5% agar) for plasmid construction and strain maintenance. All of the induction studies were done using M9 minimal media supplemented with 6.8 g/L Na_2PO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 1 g/L NH_4Cl (all from Sigma-Aldrich, St. Louis, MO, USA), 2 mM $MgSO_4$ (Fischer Scientific), 100 μM $CaCl_2$ (Sigma-Aldrich), 0.4% glucose (Sigma-Aldrich), and 0.2% casamino acids (Acros Organics, Geel, Belgium). Both the LB and M9 media were supplemented with 20 μg/mL of chloramphenicol (Acros Organics), 25 μg/mL ampicillin (Acros Organics), and appropriate concentrations of isopropyl–D-1-thiogalactopyranoside (IPTG) (USB Molecular Biology Reagents/Affymetrix, Santa Clara, CA, USA), or L (+)-arabinose (Sigma-Aldrich). Phosphate-buffered saline (PBS) with 2 mg/mL kanamycin was utilized throughout the studies.

Molecular biology reagents

Restriction enzymes, T4 polynucleotide kinase, and T4 DNA ligase used in this study were purchased from New England Biolabs (Frankfurt, Germany). Plasmid DNA preparations were made by using the oligo-linker mediated assembly (OLMA) method [40]. Primers and short segments of single-stranded DNA (oligonucleotides) were purchased from BGI (Beijing Genomics Institute, Beijing, China). Plasmid DNA concentrations were measured using a Nano Drop® ND-2000 spectrophotometer (Peqlab, Erlangen, Germany).

Staining and plasmids

All plasmid constructs were transformed into and maintained in *Escherichia coli* strain DH5α (TransGen Biotech, Beijing, China). The *E. coli* stain T7E1a was previously developed by our lab by integrating the T7 RNA polymerase into *E. coli* DH10B (TransGen Biotech) using the pOSIP vector [40] and was used as the test strain. The OLMA method was used for plasmid construction throughout the study unless otherwise specified. Two vectors were previously constructed by our lab for procedural DNA assembly, the Repressor Generator Plasmid (RGP) and the Promoter Tester Plasmid (PTP), as described in our previous report [41]. The RGP was designed as the vector for a series of plasmids using IPTG-induced expression of different repressors. In addition to the p15A origin, the Amp^R and *LacI* genes were overexpressed by a constitutive promoter, and two BsaI sites flanked a LacZ alpha fragment and was positioned such that the recognition sites were eliminated from the vector after digestion. Using the Golden Gate cloning reaction, the RGP series of plasmids were constructed with the LacZ alpha fragment replaced by the wild type or reconstructed repressors of interest. The PTP was prepared to characterize the strength of the promoters with different operators. In addition to pSC101 and Cm^R, the PTP also had two BsaI sites flanking a LacZ alpha fragment, which could be

eliminated from the vector after digestion to insert the promoters of interest by substituting the LacZ alpha fragment. The promoters were constructed by total synthesis and ligation.

Cell growth conditions

The cells were grown according to the following protocol before assaying their fluorescence, as reported previously[23]. First, cells were inoculated from single colonies on LB agar plates and grown overnight in 4 mL of LB liquid media in Falcon tubes at 37°C with shaking (250 rpm). Then, the overnight cultures were diluted 200-fold into prewarmed M9 media in 96-well plates. The plates were then incubated at 37°C with orbital shaking, and the optical density at 600 nm was recorded every five min using a Safire plate spectrophotometer (Tecan, San Jose, CA, USA) Once the diluted cultures reached an OD600 of 0.12–0.14 (~3 h), the cultures were diluted 700 fold and placed into 96-well plates with prewarmed M9 media containing the inducer. These new plates were incubated at 37°C in a Digital Thermostatic Shaker (Elmi, Riga, Latvia), with shaking at 1,000 rpm for 6 h to maintain exponential growth. Finally, after the 6-h induction, a sample of each culture was transferred to a new plate containing 150 mL of PBS and 2 mg/mL of kanamycin, to prevent protein synthesis.

Fluorescence measurements

The fluorescence distribution of each sample was measured using a LSRII flow cytometry system (BD Biosciences, San Jose, CA, USA) with appropriate voltage settings (FSC:516, SSC:286, FITC:680, B:650). Each sample contained at least 50,000 cells and was gated by the forward and side scattering using FlowJo, version 7.6 (Ashland, OR, USA). The geometric mean of each sample was calculated. The autofluorescence of *E. coli* DH10B cells with negative-control plasmids alone was measured and subtracted from the mean value to generate the reported values of fluorescence.

Data analysis

The GFP expression level was calculated as follows:

$$F = \beta + (V_{\max} - \beta) \cdot \frac{K^n}{K^n + R^n} \qquad (1)$$

Where R refers to the number of repressor proteins, and K is the *in vivo* dissociation constant for a repressor from the promoter. The relationship of F versus R is an inverse sigmoid curve (Hill function). When compared with the experimental results of the fluorescence measurements, V_{max} is the GFP expression level of the original T7 promoter without the operon site, and R = 0. As measured in our study, the value of Vmax was 2388 (U). However, β is a basal expression level depending on the repressor binding-site. Fitting the experimental data to equation 1, and allowing K and n to vary simultaneously, the repression quality or response sensitivity was characterized by a Hill coefficient

(n). Least-square fitting was used to produce quantitative curves as close as possible to the experimental results, thus characterizing the repressive quality of each reconstructed repressor. Using the function *fminsearch* of matlab, the parameters were determined by searching the minimal solution of a sum through one data set:

$$\sum \left(\frac{Experimental - Theoretical}{Experimental} \right)^2 \qquad (2)$$

All data fitting and the data plotting of experimental results were performed using MATLAB, version 7.11.0.584 (R2010b) (Mathworks, Sunnyvale, CA, USA).

Supporting Information

Figure S1-3 supporting the results in the main text; Table S1-4 including the sequences of the used genetic parts and the parameters for model fitting.

Acknowledgements

This study was supported by the NSFC (31470818 and 31722002), the MSTC (2015CB910300), CAS Interdisciplinary Innovation Team (No. Y429012CX8), and the CAS/SAFEA International Partnership Program for Creative Research Teams.

Author contributions

C.L. conceived and supervised the project. J.H., Y.Z., Z.C. and C.M. designed and performed the experiments. W.Z. and C.L. constructed the biophysical model. W.Z., B.W., and C.L. wrote or revised the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Cuthbertson, L., and Nodwell, J. R. (2013) The TetR family of regulators, *Microbiology and molecular biology reviews : MMBR 77*, 440-475.
2. Levine, M., Cattoglio, C., and Tjian, R. (2014) Looping back to leap forward: transcription enters a new era, *Cell 157*, 13-25.
3. Ang, J., Harris, E., Hussey, B. J., Kil, R., and McMillen, D. R. (2013) Tuning response curves for synthetic biology, *Acs Synth Biol 2*, 547-567.
4. Dodd, I. B., Shearwin, K. E., Perkins, A. J., Burr, T., Hochschild, A., and Egan, J. B. (2004) Cooperativity in long-range gene regulation by the lambda CI repressor, *Genes Dev 18*, 344-354.
5. Shis, D. L., Hussain, F., Meinhardt, S., Swint-Kruse, L., and Bennett, M. R. (2014) Modular, multi-input

transcriptional logic gating with orthogonal LacI/GalR family chimeras, *Acs Synth Biol 3*, 645-651.

6. Zhan, J., Ding, B., Ma, R., Ma, X., Su, X., Zhao, Y., Liu, Z., Wu, J., and Liu, H. (2010) Develop reusable and combinable designs for transcriptional logic gates, *Molecular systems biology 6*, 388.
7. Collins, C. H., Arnold, F. H., and Leadbetter, J. R. (2005) Directed evolution of Vibrio fischeri LuxR for increased sensitivity to a broad spectrum of acyl-homoserine lactones, *Mol Microbiol 55*, 712-723.
8. Taylor, N. D., Garruss, A. S., Moretti, R., Chan, S., Arbing, M. A., Cascio, D., Rogers, J. K., Isaacs, F. J., Kosuri, S., Baker, D., Fields, S., Church, G. M., and Raman, S. (2016) Engineering an allosteric transcription factor to respond to new ligands, *Nat Methods 13*, 177-183.
9. Tang, S. Y., Qian, S., Akinterinwa, O., Frei, C. S., Gredell, J. A., and Cirino, P. C. (2013) Screening for enhanced triacetic acid lactone production by recombinant Escherichia coli expressing a designed triacetic acid lactone reporter, *J Am Chem Soc 135*, 10099-10103.
10. Ali, M. H., and Imperiali, B. (2005) Protein oligomerization: How and why, *Bioorgan Med Chem 13*, 5013-5020.
11. Funnell, A. P. W., and Crossley, M. (2012) Homo- and Heterodimerization in Transcriptional Regulation, *Adv Exp Med Biol 747*, 105-121.
12. Gaglia, G., Guan, Y., Shah, J. V., and Lahav, G. (2013) Activation and control of p53 tetramerization in individual living cells, *Proceedings of the National Academy of Sciences of the United States of America 110*, 15497-15501.
13. Priest, D. G., Cui, L., Kumar, S., Dunlap, D. D., Dodd, I. B., and Shearwin, K. E. (2014) Quantitation of the DNA tethering effect in long-range DNA looping in vivo and in vitro using the Lac and lambda repressors, *Proceedings of the National Academy of Sciences of the United States of America 111*, 349-354.
14. Knapp, G. S., and Hu, J. C. (2010) Specificity of the E. coli LysR-type transcriptional regulators, *Plos One 5*, e15189.
15. Vilar, J. M., and Leibler, S. (2003) DNA looping and physical constraints on transcription regulation, *J Mol Biol 331*, 981-989.
16. Camsund, D., Heidorn, T., and Lindblad, P. (2014) Design and analysis of LacI-repressed promoters and DNA-looping in a cyanobacterium, *Journal of biological engineering 8*, 4.
17. Lou, C., Liu, X., Ni, M., Huang, Y., Huang, Q., Huang, L., Jiang, L., Lu, D., Wang, M., Liu, C., Chen, D., Chen, C., Chen, X., Yang, L., Ma, H., Chen, J., and Ouyang, Q. (2010) Synthesizing a novel genetic sequential logic circuit: a push-on push-off switch, *Molecular systems biology 6*, 350.
18. Liu, D., Xiao, Y., Evans, B. S., and Zhang, F. (2015) Negative feedback regulation of fatty acid production based on a malonyl-CoA sensor-actuator, *Acs Synth Biol 4*, 132-140.
19. Xu, P., Wang, W., Li, L., Bhan, N., Zhang, F., and Koffas, M. A. (2014) Design and kinetic analysis of a hybrid promoter-regulator system for malonyl-CoA sensing in Escherichia coli, *Acs Chem Biol 9*, 451-458.
20. Whipple, F. W., Hou, E. F., and Hochschild, A. (1998) Amino acid-amino acid contacts at the cooperativity interface of the bacteriophage lambda and P22 repressors, *Genes Dev 12*, 2791-2802.
21. Bell, C. E., Frescura, P., Hochschild, A., and Lewis, M. (2000) Crystal structure of the lambda repressor

C-terminal domain provides a model for cooperative operator binding, *Cell 101*, 801-811.

22. Cui, L., Murchland, I., Shearwin, K. E., and Dodd, I. B. (2013) Enhancer-like long-range transcriptional activation by lambda CI-mediated DNA looping, *Proceedings of the National Academy of Sciences of the United States of America 110*, 2922-2927.
23. Lou, C., Stanton, B., Chen, Y. J., Munsky, B., and Voigt, C. A. (2012) Ribozyme-based insulator parts buffer synthetic circuits from genetic context, *Nature biotechnology 30*, 1137-1142.
24. Kedzierska, B., Lian, L. Y., and Hayes, F. (2007) Toxin-antitoxin regulation: bimodal interaction of YefM-YoeB with paired DNA palindromes exerts transcriptional autorepression, *Nucleic acids research 35*, 325-339.
25. Marianovsky, I., Aizenman, E., Engelberg-Kulka, H., and Glaser, G. (2001) The regulation of the Escherichia coli mazEF promoter involves an unusual alternating palindrome, *J Biol Chem 276*, 5975-5984.
26. Ptashne, M., Jeffrey, A., Johnson, A. D., Maurer, R., Meyer, B. J., Pabo, C. O., Roberts, T. M., and Sauer, R. T. (1980) How the lambda repressor and cro work, *Cell 19*, 1-11.
27. Palanca, C., and Rubio, V. (2016) Structure of AmtR, the global nitrogen regulator of Corynebacterium glutamicum, in free and DNA-bound forms, *FEBS J 283*, 1039-1059.
28. Abbas, A., Morrissey, J. P., Marquez, P. C., Sheehan, M. M., Delany, I. R., and O'Gara, F. (2002) Characterization of interactions between the transcriptional repressor PhlF and its binding site at the phlA promoter in Pseudomonas fluorescens F113, *J Bacteriol 184*, 3008-3016.
29. Albanesi, D., and de Mendoza, D. (2016) FapR: From Control of Membrane Lipid Homeostasis to a Biotechnological Tool, *Front Mol Biosci 3*, 64.
30. Bogosian, G., Bertrand, K., and Somerville, R. (1981) Trp repressor protein controls its own structural gene, *J Mol Biol 149*, 821-825.
31. Choi, Y. J., Morel, L., Le Francois, T., Bourque, D., Bourget, L., Groleau, D., Massie, B., and Miguez, C. B. (2010) Novel, versatile, and tightly regulated expression system for Escherichia coli strains, *Appl Environ Microbiol 76*, 5058-5066.
32. Schaerli, Y., Munteanu, A., Gili, M., Cotterell, J., Sharpe, J., and Isalan, M. (2014) A unified design space of synthetic stripe-forming networks, *Nat Commun 5*, 4905.
33. Bradley, R. W., Buck, M., and Wang, B. (2016) Recognizing and engineering digital-like logic gates and switches in gene regulatory networks, *Curr Opin Microbiol 33*, 74-82.
34. Tamsir, A., Tabor, J. J., and Voigt, C. A. (2011) Robust multicellular computing using genetically encoded NOR gates and chemical 'wires', *Nature 469*, 212-215.
35. Wang, B., Kitney, R. I., Joly, N., and Buck, M. (2011) Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology, *Nat Commun 2*, 508.
36. Nielsen, A. A., and Voigt, C. A. (2014) Multi-input CRISPR/Cas genetic circuits that interface host regulatory networks, *Molecular systems biology 10*, 763.
37. Castang, S., and Dove, S. L. (2010) High-order oligomerization is required for the function of the H-NS family member MvaT in Pseudomonas aeruginosa, *Mol Microbiol 78*, 916-931.
38. Albright, R. A., and Matthews, B. W. (1998) Crystal structure of lambda-Cro bound to a consensus operator at 3.0 A resolution, *J Mol Biol 280*, 137-151.
39. Newlove, T., Konieczka, J. H., and Cordes, M. H. (2004) Secondary structure switching in Cro protein

evolution, *Structure 12*, 569-581.

40. Jiang, W., Zhao, X., Gabrieli, T., Lou, C., Ebenstein, Y., and Zhu, T. F. (2015) Cas9-Assisted Targeting of CHromosome segments CATCH enables one-step targeted cloning of large gene clusters, *Nat Commun 6*, 8101.
41. Zong, Y., Zhang, H. M., Lyu, C., Ji, X., Hou, J., Guo, X., Ouyang, Q., and Lou, C. (2017) Insulated transcriptional elements enable precise design of genetic circuits, *Nat Commun 8*, 52.

Figure legends

Figure 1. **The design and evaluation of the engineered oligomeric domains.** (A) A diagram of the design of the oligomeric domains. The rectangle on the bottom represents the operator binding by transcription factors, and two operators can facilitate two oligomeric transcription factors to form a DNA loop around the regulated promoter. (B) The dose-response curves of the wild type (WT) CymR and three engineered CymR repressors with oligomeric domains from LacI (gray line), CI (blue line), and CI434 (red line), respectively. The "au" in the figure means arbitrary unit. (C) The dose-response curves of the engineered CymR linker, $CI434_{oligomer}$, with different numbers of the "GGGGS" peptide as the flexible linkers (black line, no linker; blue line, one copy of "GGGGS," red line, two copies of "GGGGS"). Data points indicate the means, and error bars denote the standard deviations from three independent replicates.

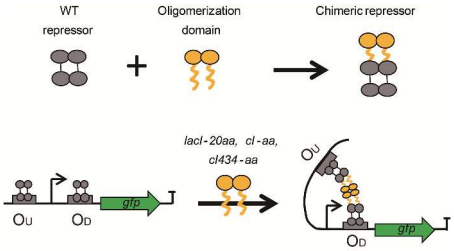
Figure 2. Generalization of the designed $CI434_{oligomer}$ oligomeric domain. (A) The dose-response curve for the oligomeric $LmrA^*$ repressor regulated on the dual O_U-O_D operators (red line) compared with the controlled ones without the oligomeric domain (blue line), or only with the single-operator (black line). (B, C) Comparing the dual O_U-O_D operators with the single O_D operator (B) or the O_{dist}+O_U-O_D tetra-operators (C) to evaluate the effects of the number of operators on engineered $LmrA^*$ regulation. (D) The evaluation of the oligomeric domain and dual operators on the cooperativity for seven transcription factors (PhlF, AmtR, CymR, TrpR, MazE, YefM, and Cro). Error bars indicate standard deviations from three independent replicates. The inserted numbers indicate the Hill coefficients of each response curve.

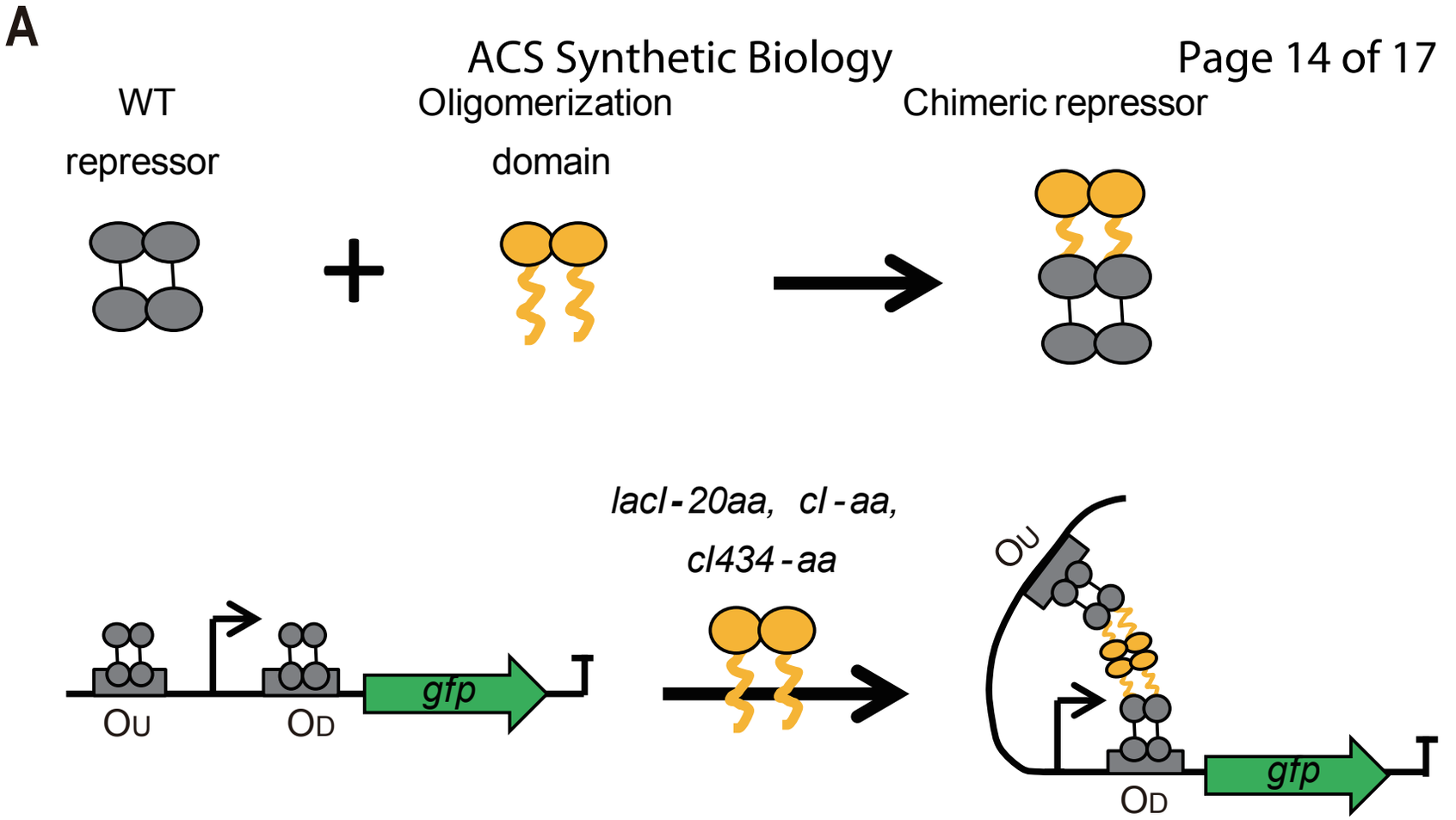
Figure 3. Modularity of the oligomeric domain on the ligand-responsive transcription factors. (A) The response curves of the regulated promoters as a function of cumate concentration, which inactivated the DNA-binding ability of the CymR (blue line) and $CymR^*$ (red line) repressor. (B) The response curves of the regulated promoters as a function of the 2,4-diacetylphoroglucinol (DAPG) concentration, which inactivated the DNA-binding ability of the PhlF (blue line) and $PhlF^*$ (red line) repressor. Data points indicate the means, and error bars denote the standard deviations from three independent replicates.

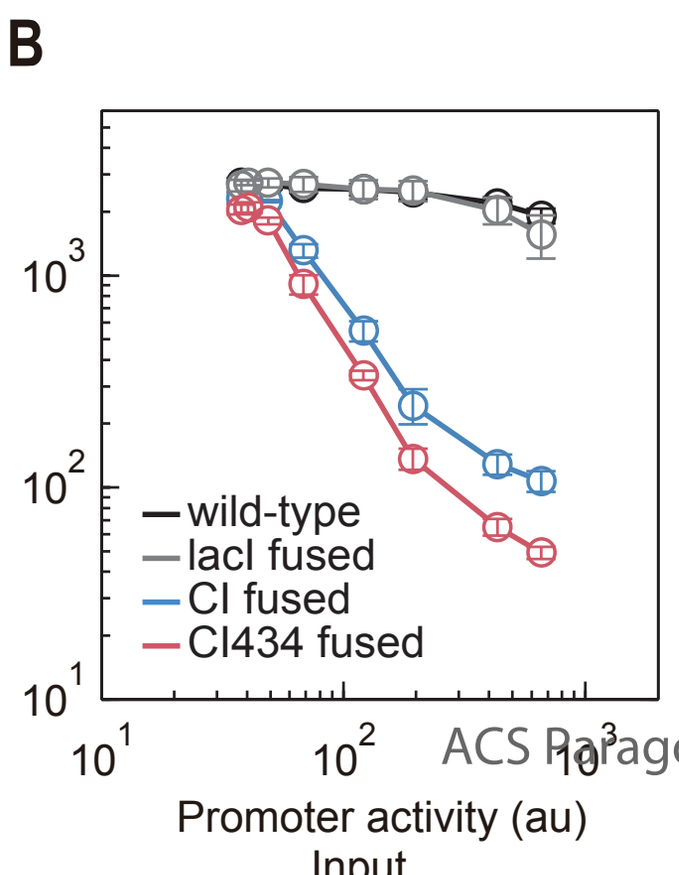
Figure 4. Improvement of the performance of the gene circuit by the oligomeric domain. (A) The design of the incoherent feedforward loop (IFFL) circuits with the WT LmrA (bottom panel) or the

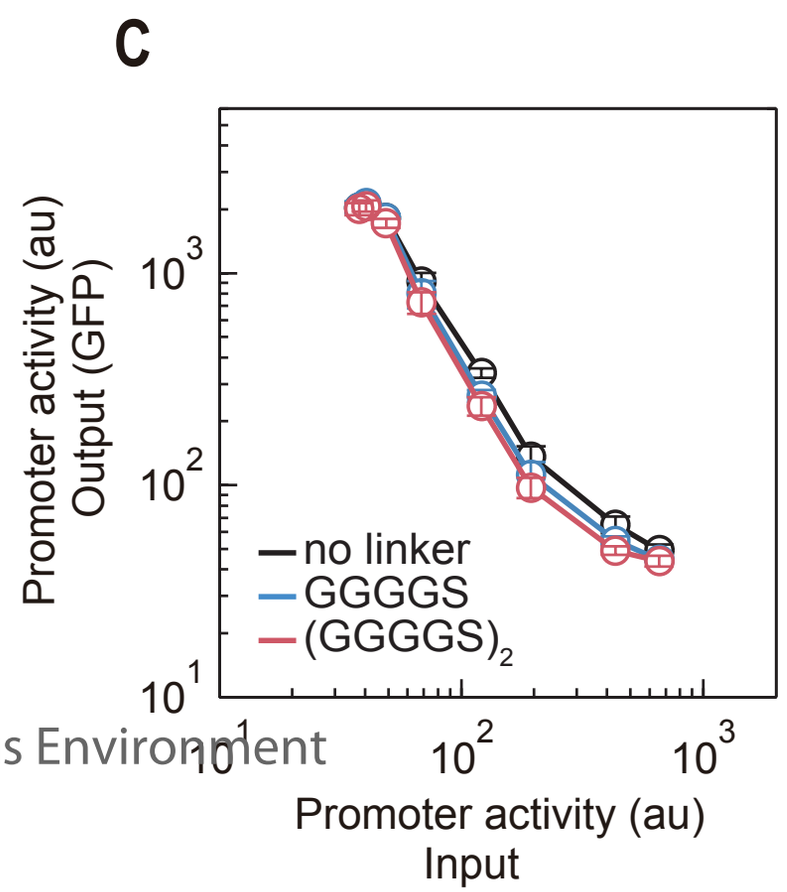
engineered LmrA* (top panel) as the repressor node. (B) The pulsed output of the IFFL circuits as a function of the input isopropyl–D-1-thiogalactopyranoside (IPTG) gradient. The blue line is the IFFL circuit with the WT LmrA repressor, while the red line is the one for the engineered LmrA*. Error bars denote standard deviations from three independent replicates.

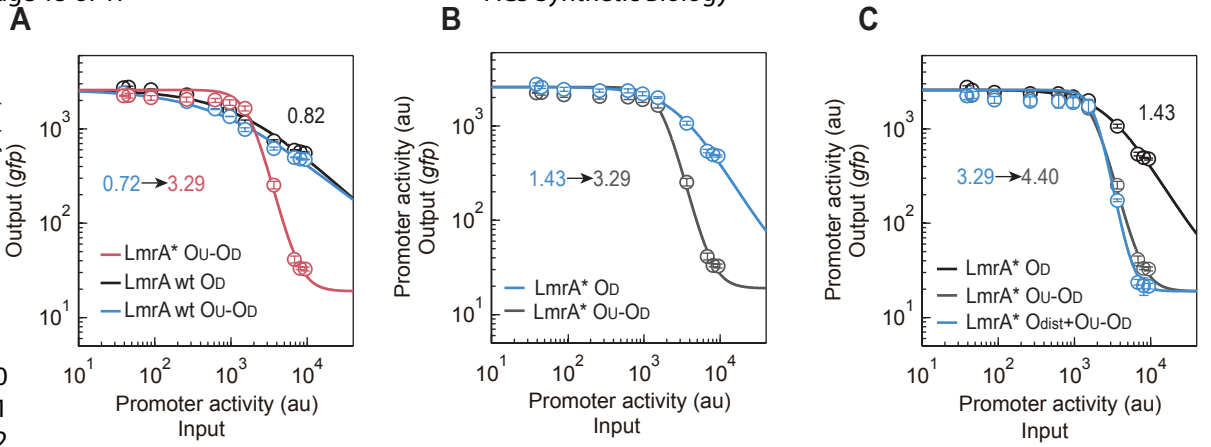
For Table of Contents Only

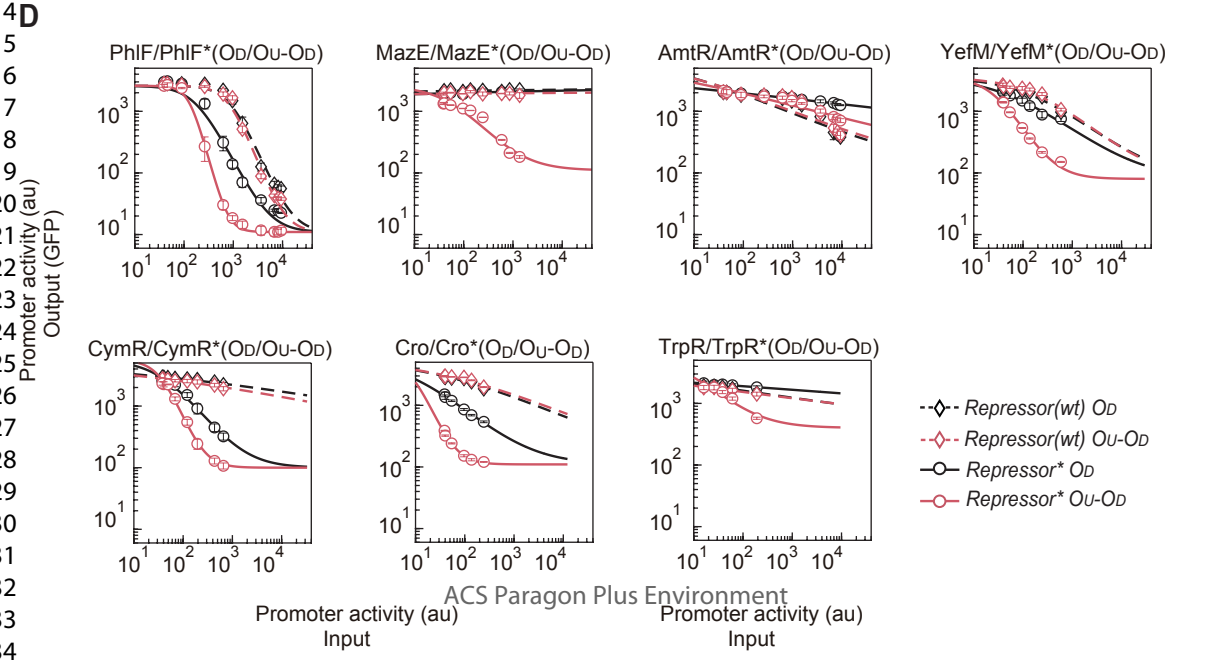


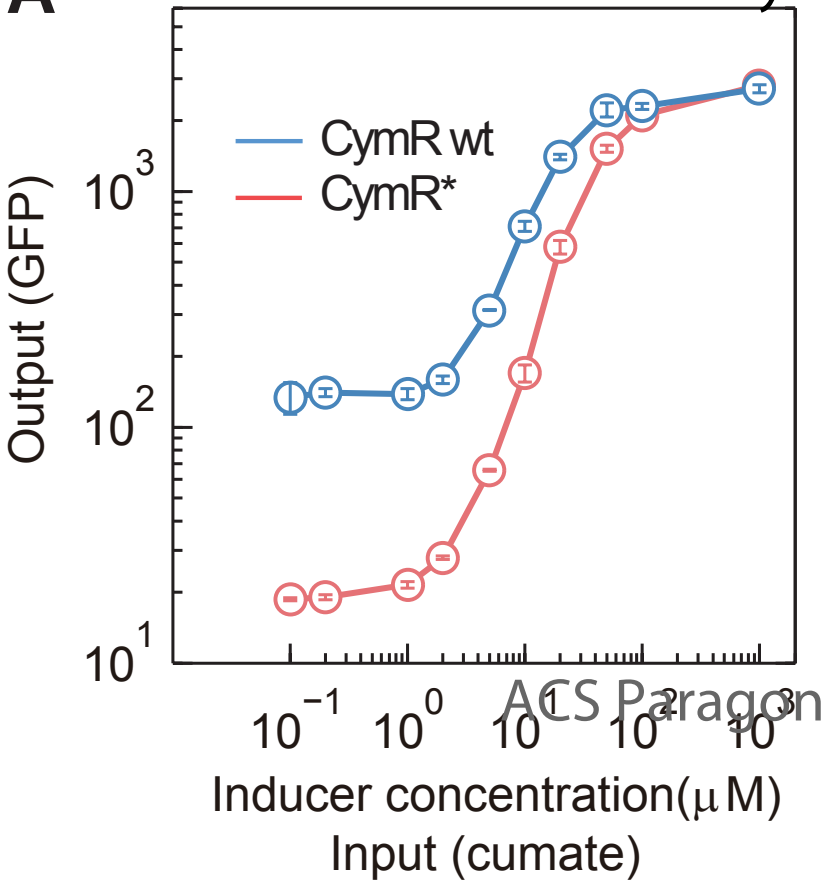


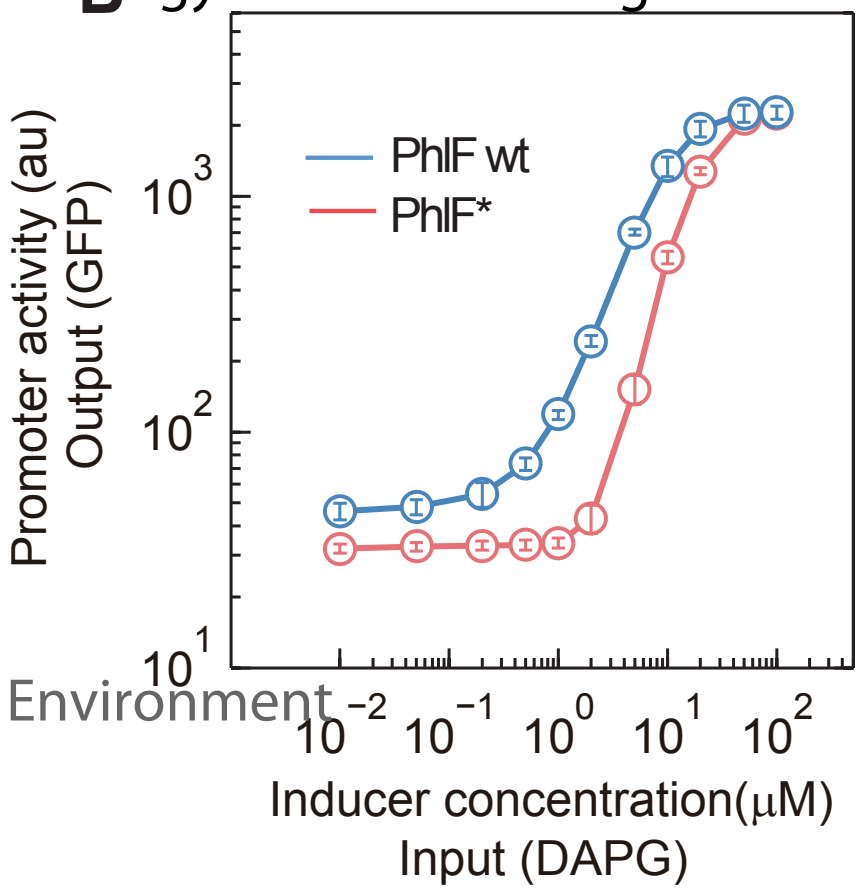




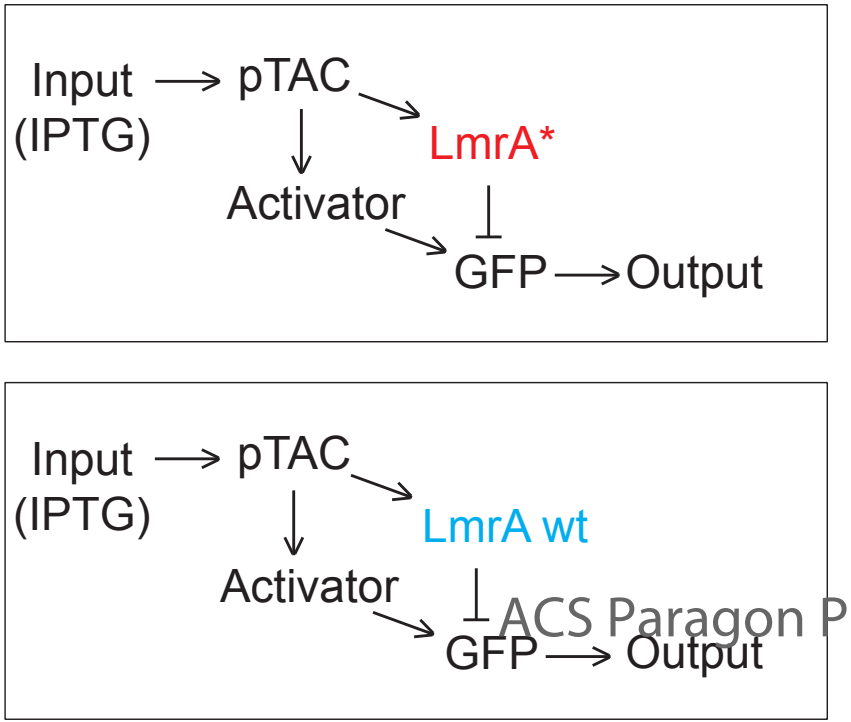








A Incoherent Feedforward Loop (I1-FFL) networks



B