Regulation by competition: a hidden layer of gene regulatory network

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Abstract

Molecular competition is ubiquitous, essential and multifunctional throughout diverse biological processes. By abstracting the analogous competition mechanism shared by diverse molecular systems, we built a unified coarse-grained competition motif model to systematically compare experimental evidences in these processes and analyzed general properties shared behind them. We could predict in what molecular environments competition would reveal threshold behavior or display a negative linear dependence. We quantified how competition can shape regulator-target dose-response curve, modulate dynamic response speed, control target expression noise, and introduce correlated fluctuations between targets. This work uncovered the complexity and generality of molecular competition effect, which might act as a hidden regulatory

mechanism with multiple functions throughout biological networks in both natural and synthetic systems.

Keywords

systems biology | computational modelling | molecular competition regulation | synthetic biology | network motif

Significance

Competition for limited resources is ubiquitous in biological processes, playing as a hidden regulatory mechanism with diverse functions. We built a unified coarse-grained competition motif model to quantitatively understand and predict diverse phenomena mediated by molecular competition. We systematically analyzed the properties of competing regulation from steady-state behavior to dynamic responses, evaluating how competition introduces indirect regulations and constraints among the targets and how the existence of competitors could influence regulator-target response. These properties provide new insights to understand natural biological systems, and can help predict and refine the performance of synthetic gene circuits.

Introduction

Competition for limited resources matters at all scales of biology. Competition among different species can alter population distributions and ecological niches (1-3). Competition among individuals of the same species may slow down the growth rates of both competitors, driving natural selection and evolution (4-6). Competition among adjacent cells in an organism can regulate their growth and viability, and enhance the dominance of cells with better fitness (7-10). In a microscopic scale, biological molecules within cells also face competition. Competition brings about trade-offs of shared limited resources among the cellular components (11-13), and it thus introduces a hidden layer of regulatory mechanism by connecting components even without direct physical interactions. Miscellaneous phenomena caused by molecular competition have been reported in a variety of biological processes in diverse organisms. For example, DNA binding sites on plasmids can compete for transcription factor (TF) LacI to dictate its target gene expression in *E. coli* (14). Noncoding RNAs transcribed from enhancer or promoter region can competitively bind to TF Yin-Yang 1 to trap the TF locally thus maintain gene expression stability in mouse embryonic stem cells (15). mRNA, long-noncoding RNA and circular RNA molecules can competitively bind to microRNAs (miRNAs) to regulate various processes, such as cell growth (16), cell differentiation (17) and tumor suppression (18). Competition between RNA binding proteins PGL-3 and MEX-5 for mRNA drives polar positioning of phase-separated liquid compartments in *C. elegans* embryos (19). Furthermore, competition effects are especially important in synthetic gene circuits. Every synthetic gene inevitably competes for common

resources with each other in circuits and with endogenous biological processes, introducing unexpected circuit failures or host metabolic burdens (20-22). In addition, when one genetic element drives two or more downstream elements, competition will modulate the dynamics of signal transduction (23, 24). As a result, characteristics of each single component are insufficient for the accurate prediction of the whole circuit behavior, posing a serious obstacle in synthetic circuit design and application.

Several mathematical frameworks and synthetic gene experiments have been built to quantitatively understand the diverse biological phenomena caused by competition. For example, a thermodynamic model was used to explain the TF titration effect in *E. coli* (14). Kinetic model has been adopted to analyze competing endogenous RNA (ceRNA) regulation (25), and we further quantified the ceRNA effect through synthetic gene circuits in human cell line (26). A minimal model based on delay differential equations was established to describe ribosome allocation between endogenous and synthetic genes in *E. coli* (27). Queueing theory was introduced to describe the protein degradation process in *E. coli*, where target proteins as queues compete for degradation machine ClpXP as server (28, 29). However, common properties and underlying competition mechanisms in essence behind these diverse phenomena have not been systematically analyzed yet.

Here we propose that regulations by competition are ubiquitous, essential and multifunctional through diverse biological regulatory processes. By abstracting the analogous competition motif shared by diverse molecular systems, we built a unified coarse-grained kinetic model to systematically summarize experimental evidences in

diverse biological processes and analyzed the common properties shared among them. We organized these properties from steady-state behavior to dynamic responses, to quantify how competition could introduce constraints and indirect regulations among the targets and how the existence of competitors might influence regulator-target response characteristics. This work demonstrated the complexity and generality of the molecular competition effect, which is a ubiquitous hidden regulatory mechanism with diverse functions throughout different biological processes in both natural and synthetic life systems.

Results

A unified coarse-gained competition motif model

A number of phenomena caused by molecular competition have been reported in diverse biological systems recently (14-16, 19). Do they share any common properties? Could they be described by a unified model? We summed up several representative competition scenarios following the life cycle of gene expression (Fig. 1), including competitions for transcription factors by DNA binding sites (Fig. 1B), competitions for miRNAs and ribosomes by RNA molecules (Figs. 1C and 1D), and competitions for degradation enzymes by target proteins (Fig. 1E). Inspired by previous models studying ceRNA effect (25, 26), we proposed a generalized competition motif model, in which two target molecule species (target#1 and #2, T_1 and T_2) competitively bind with a shared regulatory molecule species (regulator, R) (Fig. 1A), to describe the similar competition topology these cases share. In this model, each molecule species is

produced and degraded with certain rates, and the regulator is dynamically bound to targets following biochemical mass-action laws to form complexes (Fig. 1F, SI Material and Methods). Loss rates of regulator (α) and its competing targets (β) were introduced to describe reactions from pure stoichiometric ($\alpha \sim 1, \beta \sim 1$) to pure catalytic ($\alpha \sim 1, \beta \sim 0$ where enzymes act as competitors, or $\alpha \sim 0, \beta \sim 1$ when substrates act as competitors) (25). In different biochemical scenarios, experimentally measured signals may reflect different component levels of the competition motif. For example, the activity of targets could be mainly reflected by the abundance of complexes (T^{C}) when the regulator is an activator, or by the abundance of the free targets (T^{F}) when the regulator is a repressor.

This unified model can describe competitions in various biological processes (Fig. S1A-D). Despite of different parameter settings, all these cases share the core competition motif structure, suggesting that they may share common characteristics. In the following sections, we used this model to analyze, in the scenario of either steady-state behavior or dynamic response, how the competition introduces indirect regulations between targets and how the existence of the competitors influences the property of regulator-target response.

Relative abundance determines the regulatory pattern between competitors

Competition can cause crosstalk between targets. By quantifying the competition effect of one target upon the abundance of another target, recent studies have reported two apparently different steady-state behaviors named “threshold behavior” of ceRNA regulation in mammalian cells (25) and “negative linear dependence” behavior of

synthetic gene expression in bacteria (30, 31). How could competition generate such two vastly different phenomena?

The model predicted that the relative abundance between regulator and target determines the diverse behaviors. Figure 2A and S2A illustrates how molecular abundance changes along with the gradual increment of T_2's production rate. The system went through three regimes: "*R* abundant", "*R* near-equimolar" and "*R* scarce", which are mainly determined by the production rate and loss rate of each component (SI Material and Methods). In the "*R* abundant" regime, free T_1 level (T_1^F) is not sensitive to the increment of free T_2 level (T_2^F), but when the system enters the "*R* near-equimolar" regime, T_1^F becomes more sensitive to T_2^F changes, thus generates the threshold behavior (Fig. 2B and S2B). In contrast, T_1 complex level (T_1^C) is substantially unchanged with respect to T_2 complex level (T_2^C) except in the "*R* scarce" regime, where T_1^C displays a negative linear dependence with T_2^C (Fig. 2C).

In the case of ceRNA regulation, where miRNA is a repressor, target activity can be reflected by the free mRNA level. Increments of $ceRNA_2$ (T_2^F) can raise free $ceRNA_1$ (T_1^F) level indirectly by sequestering shared miRNAs. Such derepression caused by ceRNA effect is negligible when the level of $ceRNA_2$ is far less than that of miRNA (in the "*R* abundant" regime), but becomes detectable when the level of $ceRNA_2$ is comparable to that of miRNA (in the "*R* near-equimolar" regime) (25, 26). In contrast, when the regulator is an activator, target activity can be represented by the level of complexes. Recently a phenomenon called "isocost line" behavior, originally studied in economics, was also found in synthetic biological systems (30, 31) that the

expressions of two fluorescent proteins in *E. coli* displayed negative linear dependence, which was caused by competition for the transcription and translation resources (acting as activator) by the two synthetic genes. Due to the high expression level of these genes, the system was always restricted to the "*R* scarce" regime, thus showed negative linear dependence.

In summary, threshold behavior and negative linear dependence are two aspects generated by the same competition motif. The threshold behavior is observed when the regulator is a repressor and the system transfers from the "*R* abundant" to the "*R* near-equimolar" regime; while the negative linear dependence occurs when the regulator is an activator and the system is restricted to the "*R* scarce" regime.

Competition can shape dose-response curve

How does competition modulate the response of target to varying levels of a regulator? The dose-response curve, which quantitatively describes the magnitude of such responses, was systematically analyzed. Firstly, the dose-response curve of free T_1 (T_1^F) level to the total regulator (R) level without competition effect (without T_2) was calculated as the baseline. As expected (32), T_1^F was not sensitive to the regulator changes in the "*R* scarce" regime, but became sensitive in the "*R* near-equimolar" regime, thus forming some "threshold behavior" (black line in Fig. 3A-B). Then we analyzed how the molecular levels and the kinetic parameters of the competitor T_2 might influence the shape of the R-T_1^F dose-response curve. We first considered the case that T_1 and T_2 have the same kinetic parameters to bind R. Increments of T_2

production could elevate the maximum sensitivity to enhance the threshold behavior, and shift the position of the maximum sensitivity to a higher R level in the new "R near-equimolar" regime (Fig. 3A-B). We next fixed T_2's production rate and analyzed the influence of other kinetic parameters. The relative binding affinity was found as the key parameters to modulate the R-T_1^F dose-response curve. If T_2^C was formed slowly (small k_{2+}) or dissociated rapidly (large k_{2-}), T_2 could hardly alter the R-T_1^F response. Along with the increment of T_2 binding affinity (increasing k_{2+} or decreasing k_{2-}), T_2's competition blunted the sensitivity in the R~T_1 near-equimolar regime considering only R and T_1, meanwhile enhanced the sensitivity in the R~T_1+T_2 near-equimolar regime in the presence of T_2 (Fig. 3C-D and S3).

The model analysis is consistent with the experimental observations in diverse molecular competition scenarios reported previously. In the case of ceRNA (Fig. 1C), the RNA competitors with comparable binding affinities can enhance the maximum sensitivity and shift their positions in the miRNA-target dose-response curve, and a higher competing RNA level can cause a stronger enhancement and shift (26). Similarly, in the studies on the TF titration effect (Fig. 1B), introducing high affinity competitive binding sites can greatly shift and sharpen the response of primary target gene expression to the TF (14, 33). In contrast, in the case of buffer solutions in chemistry, for example the ammonium buffer, the weak base NH_4^+ compete with H^+ for OH^-, and NH_4^+ has a much lower binding affinity with OH^- than H^+ (Fig. S1E). When a mild change of OH^- (e.g. adding moderate amounts of NaOH or HCl) is introduced into the solution, NH_4^+ can buffer the response of free H^+ to OH^-, thus keeping pH (potential of

hydrogen) almost constant in a certain range (SI Material and Methods). In summary, introducing the competitors can shape the R-T_1^F dose-response curve. A high affinity competitor can enhance the maximum sensitivity and shift its position to a higher R level; while a low affinity competitor may buffer the response. The extents of such modulations are dictated by the abundance of competitors.

However, it should be noticed that when we considered the response curve of a free primary target to the level of a free regulator (R^F-T_1^F), the curve was not influenced by the existence of competitor at all (Fig. 3E). This is because, rather than the total regulator abundance, the free regulator abundance is the one effectively determines the kinetic reaction rate with each single target (34). Thus, responses of two or more targets to the shared regulator are mutually independent given the level of R^F, which provides an efficient way, by using R^F level as the medium, to analyze the relative regulatory efficiency among multi-targets (35). Once given the dose-response of each component (R^F-T_i^F, which could be separately measured or calculated) and the expected regulatory efficiency of a specific target, we can predict the level of all other targets immediately because they are all exposed to the same free regulator level (Fig. 3F, SI Material and Methods). Such property is especially important for designing synthetic circuits, where we know the characteristics of each single part and would like to predict the whole system's behavior when putting them together. We have applied this property to siRNA design principle: by both *in silico* simulation and experimental validation, it was found that the influence of a high off-target gene expression level could be compensated by introducing a suitable number of siRNAs, whereas off-target genes with strong binding

affinity should be avoided (26, 35). In summary, the dose-response to the free regulator level is not influenced by any competitors, therefore providing an efficient way to extract the relative response relations in multi-target networks.

Competition can delay or accelerate dynamic response

How does the existence of competitors influence the dynamic behavior of the system in response to a time-varying regulator? To answer this question, we simulated the response of a switching system with regulator level changing between "ON" and "OFF" states (Fig. 4A). On the rising edge of R's change, the existence of T_2's competition always delays the response of both T_1^F and T_1^C, because it can sequester R from binding with T_1 and may cause additional R loss via T_2^C degradation, both of which resist the increment of available R to regulate T_1. However, on the falling edge, competing can either accelerate or delay the response depending on the kinetic parameters (Fig. 4B-C and S4, SI Material and Methods). On the one hand, T_2^C dissociation could compensate R's decrease, but on the other hand, T_2^C degradation may cause R loss, and these two opposing effects can dominate the final modulation of the dynamic response. Our model showed that T_2 with a large complex degradation rate (g_2) and a regulator loss rate (α_2) could lead to a quick response by mediating more R loss (Fig. 4B); while T_2 with different binding affinities could either accelerate or delay the response under different parameter settings (Fig. 4C and S4C-F), because T_2 with a strong binding affinity can enhance both R compensation and R loss via T_2^C degradation at the same time.

Recently, it has been experimentally observed that the competition for LacI binding in *E. coli* delayed the rising edge response, but accelerated the falling edge response because of the loss of the regulator binding with targets through degradation and dilution (large α_2) (24). On the contrary, the existence of competitive binding sites for transcription factor SKN7m in *S. cerevisiae* was found to delay the response of the primary target on both the rising and the falling edges (36), which implied that the regulator might be protected from degradation when binding with targets (g_2 is small) (24, 37). In summary, competition can modulate the dynamic response of some targets to their upstream regulators. This may implicate a parameter tuning method to adjust the response dynamics in the presence of the competitors.

Competition can modify target expression noise level

Competition can modulate the sensitivity and the speed of a target response to a changing regulator, both of which are highly relevant to target fluctuation (38, 39). A natural question is how the existence of competitors may influence noise in the system? Here we took miRNA regulation as an example to analyze the noise level of protein products (SI Material and Methods). In systems without R and T_2, T_1 expression noise is derived from fluctuations in transcription, translation and degradation, and the coefficient of variance (CV) of T_1 gene expression approaches the "power law", as expected by the "$1/\sqrt{N}$ rule" proposed by Schrödinger (40). The introduction of R (miRNA) as repressor can decrease the noise of lowly expressed genes, meanwhile

generate a noise peak in the "*R* near-equimolar" regime for highly expressed genes (Fig. 5A), consistent with previous studies (41, 42).

The model also indicated that the competition effect of T_2 could modify T_1 expression noise significantly. As expected, introducing T_2 weakens *R*'s ability to suppress T_1, thus may impair the noise reduction in the low expression zone. Interestingly, in the high expression zone of T_1, T_2 with strong binding affinity with *R* may elevate T_1 noise level (Fig. 5B); while T_2 with weak binding affinity may substantially depress T_1 noise level (Fig. 5C). Therefore, comparing with the one-regulator-one-target scenario, introducing higher level of miRNAs and compensable weak competitors could reduce target expression noise at the low expression zone and suppress the noise peak introduced by miRNA at the high expression zone at the same time, thus could repress gene expression noise in a wide range (Fig. 5D). In summary, competition effects may modulate gene expression noise level, and in particular, abundant weak competitors have the capability to buffer gene expression noise globally (Fig. S5).

Competition can introduce correlated fluctuation between targets

Competition can not only modify the strength of target fluctuation, but also couple fluctuations between these targets (Fig. 6A). Dynamic analysis of the model's behavior around steady state with different molecular environments predicted that the free T_1 (T_1^F) and T_2 (T_2^F) are positively correlated (Fig. 6B), while the competitor complexes (T_1^C and T_2^C) are negatively correlated (Fig. 6C). The correlation strengths in both cases

are maximized in the "R near-equimolar" regime, and gradually decrease with the system away from the regime.

This phenomenon has been predicted as the "correlation resonance" by some previous theoretical analysis on gene translation (43) and protein degradation (28, 29). Two kinds of proteins (T_1^F and T_2^F) competing for degradation enzyme ClpXP (R) showed positive correlated fluctuation, which reached the maximum when the sum of two protein production rates approached to the ClpXP's processing capacity (28, 29). Another theoretical analysis showed that in translation process, fluctuations of mRNA-ribosome complexes (T_1^C and T_2^C) were negatively correlated (43). In summary, competition can introduce negatively correlated fluctuation between free targets and positively correlated fluctuation between complexes, and both of their strength reach the maximum in the "R near-equimolar" regime.

Regulator allocation to multiple targets

Regulators often bind more than two target species simultaneously. How will regulator be allocated to multiple target species? A system with multiple targets competing for the same regulator can be described by the set of allocation equations (Fig. 7A), where the proportion of the regulator occupied by a certain target in steady state is mainly determined by this target's abundance and its capabilities to bind to (and hence to consume) the regulator (SI Material and Methods). It was noticed that, the form of the regulator allocation equation is analogous to Kirchhoff's laws in current divider circuits, where R's production rate is analogous to the total current, the capability of T_i^C to

consume R is analogous to the ith branch current, and the capability of T_i^F to occupy R is analogous to the ith branch conductance (the reciprocal of resistance) (Fig. 7B). Therefore, electronic circuits and biological systems with competition may exhibit similar properties, such as the "negative linear dependence" behavior when resources are insufficient (in the "R scarce" regime) (31).

Such allocation equations have displayed in diverse mathematical models, such as the reaction rates of product formation in enzymatic reactions when multiple substrates competing for the same catalytic enzyme under the Michaelis-Menten kinetics (44), and the probabilities of promoter-TF binding when multiple promoters competing for the same TF under the thermodynamic model (45). Meanwhile, this property has helped quantify the allocations of the transcription or the translation resources for synthetic gene circuits (22, 31). We also applied such property to predict the miRNA occupancy on each target site in a specific cell type with the miRNA and the target RNA expression levels, and significantly improved the accuracy of the miRNA target prediction (46). Those miRNAs with significant occupancy changes during tumorigenesis could serve as potent biomarkers in addition to differentially expressed miRNAs.

Discussion

Competition for limited resources is ubiquitous throughout diverse molecular reactions in both natural and synthetic biological systems. Using a coarse-gained mathematical model, we systematically analyzed the steady-state behavior and the dynamic properties of various competition network motifs, from the view of indirect regulations

among the competitors as well as the effects of the competitors on the regulator-target response (Table 1). It should be noticed that, most of the mentioned properties are connected with the concept of the *regimes* determined by the regulator-target relative abundance (Fig. 2): threshold behavior occurs when system transfers from the "*R* abundant" to the "*R* near-equimolar" regime, and linear negative dependence happens when system is in the "*R* scarce" regime; while the sensitivity of the dose-response curve, the correlated fluctuation, and the noise of the target level are all maximized in the "*R* near-equimolar" regime.

Competition motif is a common network component. It seldom functions as an isolated module in real-world biological systems, but often interacts with other components to form complex networks. For example, simulation analysis on ceRNA regulation suggested that additional targets and regulators connected with different topology could enhance or weaken the ceRNA effect (25). Theoretical analysis predicted that competition for degradation enzyme could either promote or suppress the robustness of biological oscillating circuit with different topological structures (47). In addition, competition motif could perform a variety of functions by combining with other network motifs. For example, cooperating with the positive feedback motif, competition can generate the winner-take-all (WTA) behavior (48), which have been applied to design *in vitro* molecular circuits for supervised learning and pattern classification using DNA strand displacement (49, 50).

The unified competition model gives inspirations for transferring knowledge among different molecular scenarios, since similar molecular network topology may

perform similar functions. For example, the case that ceRNA competition can sharpen the dose-response curve of miRNA regulation (26) is quite similar to that observed for TF titration effect (14). Such generality and feasibility give our confidence to make new predictions based on the competition model. For instance, the properties of pH buffer solutions demonstrated that some weak competitors could desensitize the response of the primary target to the regulator, which implies the potential role of many competitors as noise buffer. Functions of numerous miRNA target sites have long been a mystery that each miRNA species in mammalian cell could bind to hundreds target RNA species, but only a small portion of the targets with multiple high affinity binding sites could be moderately repressed (rarely exceeds 2-folds). That is to say, in most cases, miRNA binding are not functioned as intensive repression (51). Why are there so many evolutionary conserved miRNAs and potential targets if this is an inefficient regulatory mechanism? The competition model provides a possible explanation that such widespread miRNA competitors with low binding affinity could buffer noise and stabilize gene expression.

Competition effect is one of the major challenges for circuits design in synthetic biology. Synthetic gene expression can lead to intracellular resource reallocation, which may affect the performance of both exogenous gene circuits and host gene networks simultaneously. It may change the network structure of the original designed circuits by introducing a hidden layer of regulation, making it difficult to predict the whole circuit's behavior based on the characteristic of each individual component. For example, competition for cellular resources may reshape the response of genetic

activation cascades in *E. coli* (22), and multiple downstream genes competing for upstream signal molecules may accentuate the "retroactivity" (52). It has been found that the induction strength of the synthetic gene oscillator could influence the growth rate of host cell, the expression of endogenous genes, and the performance of the oscillator, such as amplification and period (12). On the other hand, interestingly, using competition effect properly to rebalance synthetic circuits' relation to the host cell is emerging as an effective way to refine circuits performance. For example, the robustness of the synthetic oscillator can be greatly improved by introducing competing binding sites for TF LacI to sharpen target gene dose response curves and suppress gene expression noise (53). Models incorporating circuit-host competition effects can predict synthetic gene behaviors better (54). Reallocating the cellular translational resources by introducing the endoribonuclease MazF circuit can significantly enhance exogenous enzyme expression to promote metabolite production (55). Utilizing synthetic miRNA and its competitive binding RNA sponges, a RNA-based AND gate circuit was designed for selectively triggering T cell-mediated killing of cancer cells (56).

As discussed in this paper, competition of molecules matters in diverse biological processes, not only convoluting regulations in cell, but also introducing plentiful functions. The concept of competition motifs and its coarse-gained model may provide a unified insight to understand diverse molecular competition phenomena, and modulate biological networks by coupling or decoupling components on the hidden layer.

Materials and Methods

Detailed information about mathematical derivations and simulations is available in SI Materials and Methods. Parameters for simulations are shown in Table S1.

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References

1. Hardin G (1960) The Competitive Exclusion Principle. *Science* 131(3409):1292-1297.
2. Schoener TW (1983) Field Experiments on Interspecific Competition. *The American Naturalist* 122(2):240-285.
3. Connell JH (1983) On the Prevalence and Relative Importance of Interspecific Competition: Evidence from Field Experiments. *The American Naturalist* 122(5):661-696.
4. Zwietering M, Jongenburger I, Rombouts F, & Van't Riet K (1990) Modeling of the bacterial growth curve. *Applied and environmental microbiology* 56(6):1875-1881.
5. Bolnick DI (2004) Can Intraspecific Competition Drive Disruptive Selection? An Experimental Test in Natural Populations of Sticklebacks. *Evolution* 58(3).
6. Svanback R & Bolnick DI (2007) Intraspecific competition drives increased resource use diversity within a natural population. *Proc Biol Sci* 274(1611):839-844.
7. Khare A & Shaulsky G (2006) First among equals: competition between genetically identical cells. *Nat Rev Genet* 7(7):577-583.
8. Johnston LA (2009) Competitive interactions between cells: death, growth, and geography. *Science* 324(5935):1679-1682.
9. Laird AK (1964) Dynamics of Tumor Growth. *British Journal of Cancer* 18(3):490-502.
10. Chang CH*, et al.* (2015) Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell* 162(6):1229-1241.
11. Scott M, Gunderson CW, Mateescu EM, Zhang Z, & Hwa T (2010) Interdependence of cell growth and gene expression: origins and consequences. *Science* 330(6007):1099-1102.
12. Weisse AY, Oyarzun DA, Danos V, & Swain PS (2015) Mechanistic links between cellular trade-offs, gene expression, and growth. *Proc Natl Acad Sci U S A* 112(9):E1038-1047.

13. Hui S, *et al.* (2015) Quantitative proteomic analysis reveals a simple strategy of global resource allocation in bacteria. *Mol Syst Biol* 11(1):784.
14. Brewster RC, *et al.* (2014) The transcription factor titration effect dictates level of gene expression. *Cell* 156(6):1312-1323.
15. Sigova AA, *et al.* (2015) Transcription factor trapping by RNA in gene regulatory elements. *Science* 350(6263):978-981.
16. Zheng Q, *et al.* (2016) Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun* 7:11215.
17. Cesana M, *et al.* (2011) A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 147(2):358-369.
18. Sumazin P, *et al.* (2011) An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. *Cell* 147(2):370-381.
19. Saha S, *et al.* (2016) Polar Positioning of Phase-Separated Liquid Compartments in Cells Regulated by an mRNA Competition Mechanism. *Cell* 166(6):1572-1584 e1516.
20. Cardinale S & Arkin AP (2012) Contextualizing context for synthetic biology--identifying causes of failure of synthetic biological systems. *Biotechnol J* 7(7):856-866.
21. Wu G, *et al.* (2016) Metabolic Burden: Cornerstones in Synthetic Biology and Metabolic Engineering Applications. *Trends Biotechnol* 34(8):652-664.
22. Qian Y, Huang HH, Jimenez JI, & Del Vecchio D (2017) Resource Competition Shapes the Response of Genetic Circuits. *ACS Synth Biol* 6(7):1263-1272.
23. Jiang P, *et al.* (2011) Load-induced modulation of signal transduction networks. *Sci Signal* 4(194):ra67.
24. Jayanthi S, Nilgiriwala KS, & Del Vecchio D (2013) Retroactivity controls the temporal dynamics of gene transcription. *ACS Synth Biol* 2(8):431-441.
25. Ala U, *et al.* (2013) Integrated transcriptional and competitive endogenous RNA networks are cross-regulated in permissive molecular environments. *Proc Natl Acad Sci U S A* 110(18):7154-7159.
26. Yuan Y, *et al.* (2015) Model-guided quantitative analysis of microRNA-mediated regulation on competing endogenous RNAs using a synthetic gene circuit. *Proc Natl Acad Sci U S A* 112(10):3158-3163.
27. Gorochowski TE, Avcilar-Kucukgoze I, Bovenberg RA, Roubos JA, & Ignatova Z (2016) A Minimal Model of Ribosome Allocation Dynamics Captures Trade-offs in Expression between Endogenous and Synthetic Genes. *ACS Synth Biol* 5(7):710-720.
28. Mather WH, Cookson NA, Hasty J, Tsimring LS, & Williams RJ (2010) Correlation resonance generated by coupled enzymatic processing. *Biophys J* 99(10):3172-3181.
29. Cookson NA, *et al.* (2011) Queueing up for enzymatic processing: correlated signaling through coupled degradation. *Mol Syst Biol* 7:561.
30. Gyorgy A, *et al.* (2015) Isocost Lines Describe the Cellular Economy of Genetic Circuits. *Biophys J* 109(3):639-646.
31. Carbonell-Ballestero M, Garcia-Ramallo E, Montanez R, Rodriguez-Caso C, & Macia J (2016) Dealing with the genetic load in bacterial synthetic biology circuits: convergences with the Ohm's law. *Nucleic Acids Res* 44(1):496-507.

32. Buchler NE & Louis M (2008) Molecular titration and ultrasensitivity in regulatory networks. *J Mol Biol* 384(5):1106-1119.
33. Lee TH & Maheshri N (2012) A regulatory role for repeated decoy transcription factor binding sites in target gene expression. *Mol Syst Biol* 8:576.
34. Jens M & Rajewsky N (2015) Competition between target sites of regulators shapes post-transcriptional gene regulation. *Nat Rev Genet* 16(2):113-126.
35. Yuan Y, Ren X, Xie Z, & Wang X (2016) A quantitative understanding of microRNA-mediated competing endogenous RNA regulation. *Quantitative Biology* 4(1):47-57.
36. Mishra D, Rivera PM, Lin A, Del Vecchio D, & Weiss R (2014) A load driver device for engineering modularity in biological networks. *Nat Biotechnol* 32(12):1268-1275.
37. Burger A, Walczak AM, & Wolynes PG (2010) Abduction and asylum in the lives of transcription factors. *Proc Natl Acad Sci U S A* 107(9):4016-4021.
38. Blake WJ, M KA, Cantor CR, & Collins JJ (2003) Noise in eukaryotic gene expression. *Nature* 422(6932):633-637.
39. Chen M, Wang L, Liu CC, & Nie Q (2013) Noise attenuation in the ON and OFF states of biological switches. *ACS Synth Biol* 2(10):587-593.
40. Schrödinger E (1944) *What is life?* (Cambridge University Press).
41. Schmiedel JM*, et al.* (2015) Gene expression. MicroRNA control of protein expression noise. *Science* 348(6230):128-132.
42. Bosia C*, et al.* (2017) RNAs competing for microRNAs mutually influence their fluctuations in a highly non-linear microRNA-dependent manner in single cells. *Genome Biol* 18(1):37.
43. Mather WH, Hasty J, Tsimring LS, & Williams RJ (2013) Translational cross talk in gene networks. *Biophys J* 104(11):2564-2572.
44. Chou T-C & Talaly P (1977) A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. *Journal of Biological Chemistry* 252(18):6438-6442.
45. Bintu L*, et al.* (2005) Transcriptional regulation by the numbers: models. *Curr Opin Genet Dev* 15(2):116-124.
46. Xie P, Liu Y, Li Y, Zhang MQ, & Wang X (2014) MIROR: a method for cell-type specific microRNA occupancy rate prediction. *Mol Biosyst* 10(6):1377-1384.
47. Rondelez Y (2012) Competition for catalytic resources alters biological network dynamics. *Phys Rev Lett* 108(1):018102.
48. Kim J, Hopfield J, & Winfree E (2004) Neural Network Computation by In Vitro Transcriptional Circuits. *Advances in Neural Information Processing Systems*, pp 681-688.
49. Genot AJ, Fujii T, & Rondelez Y (2013) Scaling down DNA circuits with competitive neural networks. *J R Soc Interface* 10(85):20130212.
50. Lakin MR & Stefanovic D (2016) Supervised Learning in Adaptive DNA Strand Displacement Networks. *ACS Synth Biol* 5(8):885-897.
51. Seitz H (2009) Redefining microRNA targets. *Curr Biol* 19(10):870-873.
52. Brophy JA & Voigt CA (2014) Principles of genetic circuit design. *Nat Methods* 11(5):508-520.

53. Potvin-Trottier L, Lord ND, Vinnicombe G, & Paulsson J (2016) Synchronous long-term oscillations in a synthetic gene circuit. *Nature* 538(7626):514-517.
54. Liao C, Blanchard AE, & Lu T (2017) An integrative circuit-host modelling framework for predicting synthetic gene network behaviours. *Nat Microbiol* 2(12):1658-1666.
55. Venturelli OS*, et al.* (2017) Programming mRNA decay to modulate synthetic circuit resource allocation. *Nat Commun* 8:15128.
56. Nissim L*, et al.* (2017) Synthetic RNA-Based Immunomodulatory Gene Circuits for Cancer Immunotherapy. *Cell* 171(5):1138-1150 e1115.

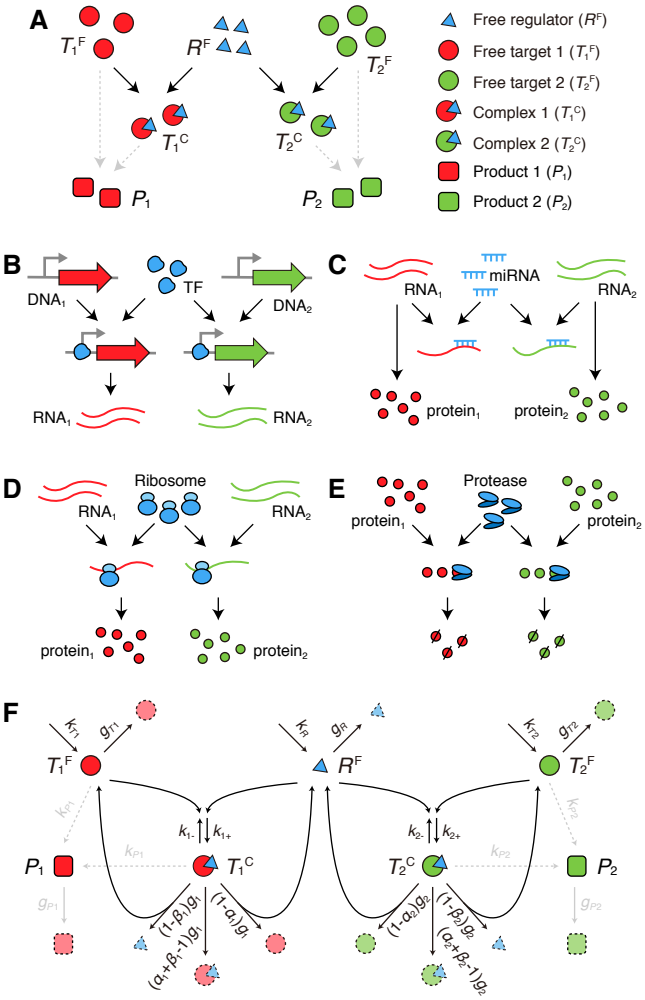

Fig. 1 Coarse-gained competition motif model. (*A*) Basic structure of the competition motif. Downstream products can be produced from either free targets or complexes. (*B*-*E*) Competition motifs abstracted from diverse competition scenarios: (*B*) DNA binding sites competing for TFs; (*C*) RNA molecules competing for miRNAs; (*D*) mRNA molecules competing for ribosomes; (*E*) proteins competing for proteases. (*F*) Unified kinetic model of the competition motif.

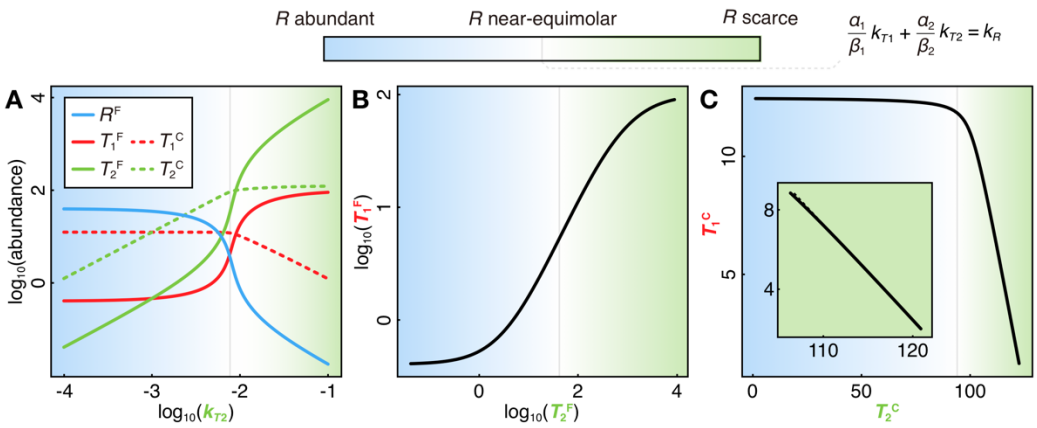

Fig. 2 Regimes of competition systems. (*A*) Abundances changes of each component with the increment of T_2's production rate (k_{T2}). (*B*) Abundance of T_1^F as a function of that of T_2^F. (*C*) Abundance of T_1^C as a function of that of T_2^C. Blue, white and green areas represent "*R* abundant", "*R* near-equimolar" and "*R* scarce" regime respectively. Grey lines represent the approximate threshold (SI Materials and Methods).

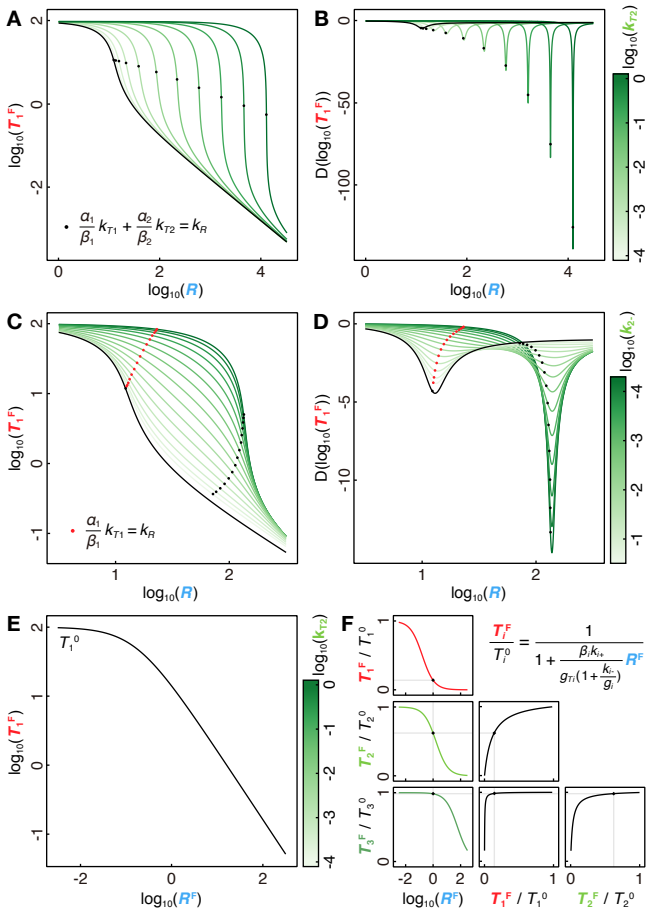

Fig. 3 Dose-response curves modulated by competition. (*A-B*) R-T_1^F dose-response curves (*A*) and their derivatives (*B*) with different T_2's production rate (k_{T2}). (*C-D*) R-T_1^F dose-response curves (*C*) and their derivatives (*D*) with different T_2^C's dissociation rate ($k_{2\text{-}}$). R represents the total abundance of regulator (R^F+T_1^C+T_2^C). Black lines represent the dose response curve without T_2 (k_{T2}=0). (*E*) R^F-T_1 dose-response curves with different k_{T2}. T_1^0 represents the abundance of T_1^F without R. Black line represents the dose response curve without T_2 (k_{T2}=0). All the curves with different k_{T2} are exactly overlapped. (*F*) Repression folds of all targets are determined by the same R^F abundance in a multi-target repression system.

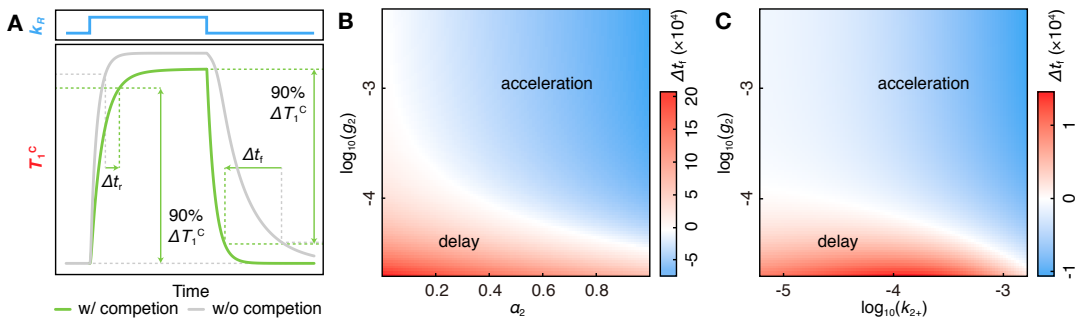

Fig. 4 Response speeds influenced by competition. (*A*) Quantitative measurements of response time. Δt_r and Δt_f represent the alteration of response time on the rising and falling edge of *R*'s change respectively. Here response time is defined as the time taken by T_1^C level to change from 0% to 90% between its initial and final steady states. (*B*-*C*) Heatmaps of Δt_f under different α_2 and g_2 (*B*), or k_{2+} and g_2 (*C*).

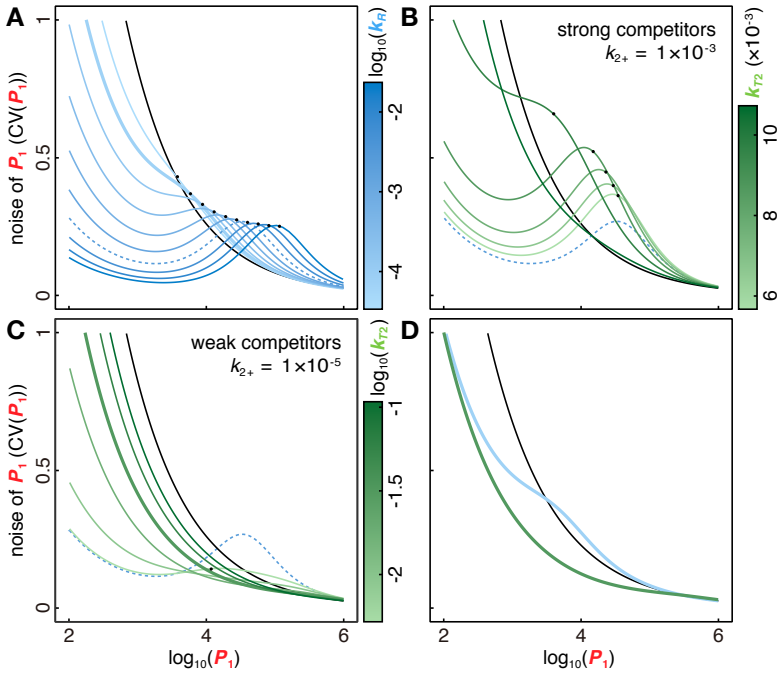

Fig. 5 Modification of target expression noise by competition. (*A*) Product expression noise (CV(P_1)) with different R's production rates (k_R). (*B*) CV(P_1) with different T_2's production rates (k_{T2}) where T_2 acts as a strong competitor. (*C*) CV(P_1) with different k_{T2} where T_2 acts as a weak competitor. (*D*) Comparison of CV(P_1) with or without competition. Here miRNA-RNA competing system is taken as an example. Black lines represent system without R. Dashed blue lines are highlighted as the basal lines in (*B*) and (*C*). The thick blue and green lines in (*D*) are taken from (*A*) and (*C*) respectively. Black dots represent the approximate threshold (there are no black dots on some curves because k_{T2} is too large to form the threshold).

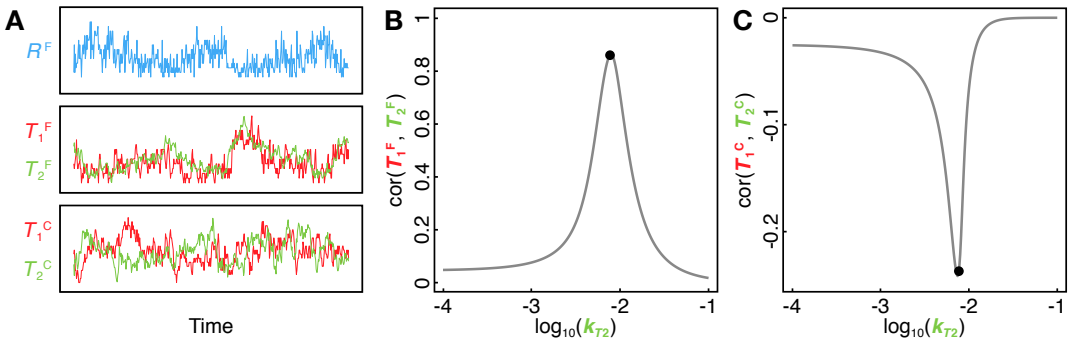

Fig. 6 Correlated fluctuations introduced by competition. (*A*) Stochastic simulations of each component's abundance in competition motif. (*B*-*C*) Correlations of T_1^F and T_2^F (*B*), or T_1^C and T_2^C (*C*) changing with T_2's production rate (k_{T2}). Black dots represent the approximate threshold.

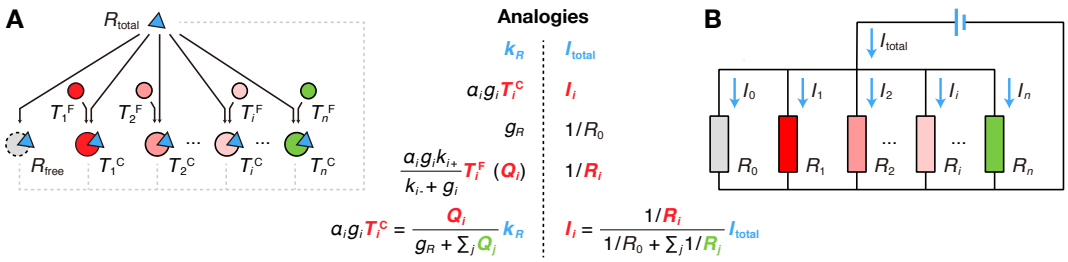

Fig. 7 Regulator allocation for multi-target competition. (*A*) Regulator allocation equations and schematic graph representation. R_{total} represent the total abundance of regulator, including free regulator and regulator in complexes. (*B*) Kirchhoff's laws in current divider circuits.

Table 1 Properties of regulation by competition

	Regulation between targets	**Influences on regulator-target response**
Steady-state behavior	Threshold behavior Negative linear dependence Regulator allocation	Shaping dose-response curves
Dynamic responses	Correlated fluctuation	Response time modulation Noise modification