Quantitative understanding of molecular competition as a hidden layer of gene regulatory network

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Abstract

Molecular competition is ubiquitous, essential and multifunctional throughout diverse biological processes. Competition brings about trade-offs of shared limited resources among the cellular components, and it thus introduce a hidden layer of regulatory mechanism by connecting components even without direct physical interactions. By abstracting the analogous competition mechanism behind 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

36 **Introduction**

37 Competition for limited resources matters at all scales of biology. Competition among
38 different species can alter population distributions and ecological niches (Connell, 1983;
39 Hardin, 1960; Schoener, 1983). Competition among individuals of the same species
40 may slow down the growth rates of all competitors, driving natural selection and
41 evolution (Bolnick, 2004; Svanback & Bolnick, 2007; Zwietering et al., 1990).
42 Competition among adjacent cells in an organism can regulate their growth and viability,
43 and enhance the dominance of cells with better fitness (Chang et al., 2015; Johnston,
44 2009; Khare & Shaulsky, 2006; Laird, 1964). In a microscopic scale, biological
45 molecules within cells also face competition. Competition brings about trade-offs of
46 shared limited resources among the cellular components (Hui et al., 2015; Scott et al.,
47 2010; Weisse et al., 2015), and it thus introduces a hidden layer of regulatory
48 mechanism by connecting components even without direct physical interactions.
49 Miscellaneous phenomena caused by molecular competition have been reported in a
50 variety of biological processes in diverse organisms. For example, DNA binding sites
51 on plasmids can compete for transcription factor (TF) LacI to dictate its target gene
52 expression in *E. coli* (Brewster et al., 2014). Noncoding RNAs transcribed from
53 enhancer or promoter region can competitively bind to TF Yin-Yang 1 to trap the TF
54 locally thus maintain gene expression stability in mouse embryonic stem cells (Sigova
55 et al., 2015). mRNA, long-noncoding RNA and circular RNA molecules can
56 competitively bind to microRNAs (miRNAs) to regulate various processes, such as cell

growth (Zheng et al., 2016), cell differentiation (Cesana et al., 2011) and tumor suppression (Sumazin et al., 2011). Competition between RNA binding proteins PGL-3 and MEX-5 for mRNA drives polar positioning of phase-separated liquid compartments in *C. elegans* embryos (Saha et al., 2016). 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 (Cardinale & Arkin, 2012; Qian et al., 2017; Wu et al., 2016). In addition, when one genetic element drives two or more downstream elements, competition will modulate the dynamics of signal transduction (Jayanthi et al., 2013; Jiang et al., 2011). 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* (Brewster et al., 2014). Kinetic model has been adopted to analyze competing endogenous RNA (ceRNA) regulation (Ala et al., 2013), and we further quantified the ceRNA effect through synthetic gene circuits in human cell line (Yuan et al., 2015). A minimal model based on delay differential equations was established to describe ribosome allocation between endogenous and synthetic genes in *E. coli* (Gorochowski

et al., 2016). 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 (Cookson et al., 2011; Mather et al., 2010). 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 integrate experimental evidences in diverse biological processes and analyze 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 (Brewster et al., 2014; Saha et al., 2016; Sigova et al., 2015; Zheng et al., 2016). 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 (Figure 1), including competitions for transcription factors by DNA binding sites (Figure 1B), competitions for miRNAs and ribosomes by RNA molecules (Figure 1C and 1D), and competitions for degradation enzymes by target proteins (Figure 1E). Inspired by previous models studying ceRNA effect (Ala et al., 2013; Yuan et al., 2015), 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) (Figure 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 (Figure 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) (Ala et al., 2013). 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

118 the abundance of complexes (T^C) when the regulator is an activator, or by the abundance
119 of the free targets (T^F) when the regulator is a repressor.
120 This unified model can describe competitions in various biological processes
121 (Figure S1A-D). Despite of different parameter settings, all these cases share the core
122 competition motif structure, suggesting that they may share common characteristics. In
123 the following sections, we used this model to analyze, in the scenario of either steady-
124 state behavior or dynamic response, how the competition introduces indirect
125 regulations between targets and how the existence of the competitors influences the
126 property of regulator-target response.
127
128 **Relative abundance determines the regulatory patterns between competitors**
129 Competition can cause crosstalk between targets. By quantifying the competition effect
130 of one target upon the abundance of another target, recent studies have reported two
131 apparently different steady-state behaviors named "threshold behavior" of ceRNA
132 regulation in mammalian cells (Ala et al., 2013) and "negative linear dependence"
133 behavior of synthetic gene expression in bacteria (Carbonell-Ballestero et al., 2016;
134 Gyorgy et al., 2015). How could competition generate such two vastly different
135 phenomena?
136 The model predicted that the relative abundance between regulator and target
137 determines the diverse behaviors. Figure 2A and S2A illustrates how molecular
138 abundance changes along with the gradual increment of T_2's production rate. The

139 system went through three regimes: "*R* abundant", "*R* near-equimolar" and "*R* scarce",
140 which are mainly determined by the production rate and loss rate of each component
141 (SI Material and Methods). In the "*R* abundant" regime, free T_1 level (T_1^F) is not
142 sensitive to the increment of free T_2 level (T_2^F), but when the system enters the "*R* near-
143 equimolar" regime, T_1^F becomes more sensitive to T_2^F changes, thus generates the
144 threshold behavior (Figure 2B and S2B). In contrast, T_1 complex level (T_1^C) is
145 substantially unchanged with respect to T_2 complex level (T_2^C) except in the "*R* scarce"
146 regime, where T_1^C displays a negative linear dependence with T_2^C (Figure 2C).

147 In the case of ceRNA regulation, where miRNA is a repressor, target activity can
148 be reflected by the free mRNA level. Increments of $ceRNA_2$ (T_2^F) can raise free $ceRNA_1$
149 (T_1^F) level indirectly by sequestering shared miRNAs. Such derepression caused by
150 ceRNA effect is negligible when the level of $ceRNA_2$ is far less than that of miRNA (in
151 the "*R* abundant" regime), but becomes detectable when the level of $ceRNA_2$ is
152 comparable to that of miRNA (in the "*R* near-equimolar" regime) (Ala et al., 2013;
153 Yuan et al., 2015). In contrast, when the regulator is an activator, target activity can be
154 represented by the level of complexes. Recently a phenomenon called "isocost line"
155 behavior, originally studied in economics, was also found in synthetic biological
156 systems (Carbonell-Ballestero et al., 2016; Gyorgy et al., 2015) that the expressions of
157 two fluorescent proteins in *E. coli* displayed negative linear dependence, which was
158 caused by competition for the transcription and translation resources (acting as activator)

159 by the two synthetic genes. Due to the high expression level of these genes, the system
160 was always restricted to the "*R* scarce" regime, thus showed negative linear dependence.
161 In summary, threshold behavior and negative linear dependence are two aspects
162 generated by the same competition motif. The threshold behavior is observed when the
163 regulator is a repressor and the system transfers from the "*R* abundant" to the "*R* near-
164 equimolar" regime; while the negative linear dependence occurs when the regulator is
165 an activator and the system is restricted to the "*R* scarce" regime.
166
167 **Competition can shape dose-response curve**
168 How does competition modulate the response of target to varying levels of a regulator?
169 The dose-response curve, which quantitatively describes the magnitude of such
170 responses, was systematically analyzed. Firstly, the dose-response curve of free T_1 (T_1^F)
171 level to the total regulator (*R*) level without competition effect (without T_2) was
172 calculated as the baseline. As expected (Buchler & Louis, 2008), T_1^F was not sensitive
173 to the regulator changes in the "*R* scarce" regime, but became sensitive in the "*R* near-
174 equimolar" regime, thus forming some "threshold behavior" (black line in Figure 2D-
175 E). Then we analyzed how the molecular levels and the kinetic parameters of the
176 competitor T_2 might influence the shape of the R-T_1^F dose-response curve. We first
177 considered the case that T_1 and T_2 have the same kinetic parameters to bind *R*.
178 Increments of T_2 production could elevate the maximum sensitivity to enhance the
179 threshold behavior, and shift the position of the maximum sensitivity to a higher *R* level

180 in the new "R near-equimolar" regime (Figure 2D-E). We next fixed T_2's production
181 rate and analyzed the influence of other kinetic parameters. The relative binding affinity
182 was found as the key parameters to modulate the R-T_1^F dose-response curve. If T_2^C was
183 formed slowly (small k_{2+}) or dissociated rapidly (large k_{2-}), T_2 could hardly alter the R-
184 T_1^F response. Along with the increment of T_2 binding affinity (increasing k_{2+} or
185 decreasing k_{2-}), T_2's competition blunted the sensitivity in the R~T_1 near-equimolar
186 regime considering only R and T_1, meanwhile enhanced the sensitivity in the R~T_1+T_2
187 near-equimolar regime in the presence of T_2 (Figure 2F-G and S2C-E).

188 The model analysis is consistent with the experimental observations in diverse
189 molecular competition scenarios reported previously. In the case of ceRNA (Figure 1C),
190 the RNA competitors with comparable binding affinities can enhance the maximum
191 sensitivity and shift their positions in the miRNA-target dose-response curve, and a
192 higher competing RNA level can cause a stronger enhancement and shift (Yuan et al.,
193 2015). Similarly, in the studies on the TF titration effect (Figure 1B), introducing high
194 affinity competitive binding sites can greatly shift and sharpen the response of primary
195 target gene expression to the TF (Brewster et al., 2014; Lee & Maheshri, 2012). In
196 contrast, in the case of buffer solutions in chemistry, for example the ammonium buffer,
197 the weak base NH_4^+ compete with H^+ for OH^-, and NH_4^+ has a much lower binding
198 affinity with OH^- than H^+ (Figure S1E). When a mild change of OH^- (e.g. adding
199 moderate amounts of NaOH or HCl) is introduced into the solution, NH_4^+ can buffer
200 the response of free H^+ to OH^-, thus keeping pH (potential of hydrogen) almost constant

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

206 However, it should be noticed that when it comes to the response curve of a free
207 primary target to the level of a free regulator (R^F-T_1^F), the curve was not influenced by
208 the existence of competitor at all (Figure 2H). This is because, rather than the total
209 regulator abundance, the free regulator abundance is the one effectively determines the
210 kinetic reaction rate with each single target (Jens & Rajewsky, 2015). Thus, responses
211 of two or more targets to the shared regulator are mutually independent given the level
212 of R^F, which provides an efficient way, by using R^F level as the medium, to analyze the
213 relative regulatory efficiency among multi-targets (Yuan et al., 2016). Once given the
214 dose-response of each component (R^F-T_i^F, which could be separately measured or
215 calculated) and the expected regulatory efficiency of a specific target, the level of all
216 other targets could be immediately predicted because they are all exposed to the same
217 free regulator level (Figure 2I, SI Material and Methods). Such property is especially
218 important for designing synthetic circuits, where we know the characteristics of each
219 single part and would like to predict the whole system's behavior when putting them
220 together. This property has been applied to siRNA design principle: by both *in silico*
221 simulation and experimental validation, we found that the influence of a high off-target

222 gene expression level could be compensated by introducing a suitable number of
223 siRNAs, whereas off-target genes with strong binding affinity should be avoided (Yuan
224 et al., 2015; Yuan et al., 2016). In summary, the dose-response to the free regulator level
225 is not influenced by any competitors, therefore providing an efficient way to extract the
226 relative response relations in multi-target networks.
227
228 **Competition can delay or accelerate dynamic response**
229 How does the existence of competitors influence the dynamic behavior of the system
230 in response to a time-varying regulator? To answer this question, we simulated the
231 response of a switching system with regulator level changing between "ON" and "OFF"
232 states (Figure 3A). On the rising edge of R's change, the existence of T_2's competition
233 always delays the response of both T_1^F and T_1^C, because it can sequester R from binding
234 with T_1 and may cause additional R loss via T_2^C degradation, both of which resist the
235 increment of available R to regulate T_1. However, on the falling edge, competing can
236 either accelerate or delay the response depending on the kinetic parameters (Figure 3B-
237 C and S3A-F, SI Material and Methods). On the one hand, T_2^C dissociation could
238 compensate R's decrease, but on the other hand, T_2^C degradation may cause R loss, and
239 these two opposing effects can dominate the final modulation of the dynamic response.
240 T_2 with a large complex degradation rate (g_2) and a large loss rate (α_2) could lead to a
241 quick response by mediating more R loss (Figure 3B); while T_2 with different binding
242 affinities could either accelerate or delay the response under different parameter settings

243 (Figure 3C and S3C-F), because T_2 with a strong binding affinity can enhance both R
244 compensation and R loss via T_2^C degradation at the same time.

245 Recently, it has been experimentally observed that the competition for LacI binding
246 in *E. coli* delayed the rising edge response, but accelerated the falling edge response
247 because of the loss of the regulator binding with targets through degradation and
248 dilution (large α_2) (Jayanthi et al., 2013). On the contrary, the existence of competitive
249 binding sites for transcription factor SKN7m in *S. cerevisiae* was found to delay the
250 response of the primary target on both the rising and the falling edges (Mishra et al.,
251 2014), which implied that the regulator might be protected from degradation when
252 binding with targets (g_2 is small) (Burger et al., 2010; Jayanthi et al., 2013). In summary,
253 competition can modulate the dynamic response of some targets to their upstream
254 regulators. This may implicate a general parameter tuning method to adjust the response
255 dynamics in the presence of the competitors.

256

257 **Competition can modify target expression noise level**

258 Competition can modulate the sensitivity and the speed of a target response to a
259 changing regulator, both of which are highly relevant to target fluctuation (Blake et al.,
260 2003; Chen et al., 2013). A natural question is how the existence of competitors may
261 influence noise in the system? Here we took miRNA regulation as an example to
262 analyze the noise level of protein products (Figure 3D, SI Material and Methods). In
263 systems without R and T_2, T_1 expression noise is derived from fluctuations in

264 transcription, translation and degradation, and the coefficient of variance (CV) of T_1
265 gene expression approaches the "power law", as expected by the "$1/\sqrt{N}$ rule"
266 proposed by Schrödinger (Schrödinger, 1944). The introduction of R (miRNA) as
267 repressor can decrease the noise of lowly expressed genes, meanwhile generate a noise
268 peak in the "R near-equimolar" regime for highly expressed genes (Figure 3E),
269 consistent with previous studies (Bosia et al., 2017; Schmiedel et al., 2015).

270 Theoretical results indicated that the competition effect of T_2 could modify T_1
271 expression noise significantly. As expected, introducing T_2 weakens R's ability to
272 suppress T_1, thus may impair the noise reduction in the low expression zone.
273 Interestingly, in the high expression zone of T_1, T_2 with strong binding affinity with R
274 may elevate T_1 noise level (Figure 3F); while T_2 with weak binding affinity may
275 substantially depress T_1 noise level (Figure 3G). Therefore, comparing with the one-
276 regulator-one-target scenario, introducing higher level of miRNAs and compensable
277 weak competitors could reduce target expression noise at the low expression zone and
278 suppress the noise peak introduced by miRNA at the high expression zone at the same
279 time, thus could repress gene expression noise in a wide range (Figure 3H). In summary,
280 competition effects may modulate gene expression noise level, and in particular,
281 abundant weak competitors have the capability to buffer gene expression noise globally
282 (Figure S3G-J).

283

284 **Competition can introduce correlated fluctuation between targets**

285 Competition can not only modify the strength of target fluctuation, but also couple
286 fluctuations between these targets (Figure 3I). Dynamic analysis of the model's
287 behavior around steady state with different molecular environments predicted that the
288 free T_1 (T_1^F) and T_2 (T_2^F) are positively correlated (Figure 3J), while the competitor
289 complexes (T_1^C and T_2^C) are negatively correlated (Figure 3K). The correlation
290 strengths in both cases are maximized in the "*R* near-equimolar" regime, and gradually
291 decrease with the system away from the regime.

292 This phenomenon has been predicted as the "correlation resonance" by some
293 previous theoretical analysis on gene translation (Mather et al., 2013) and protein
294 degradation (Cookson et al., 2011; Mather et al., 2010). Two kinds of proteins (T_1^F and
295 T_2^F) competing for degradation enzyme ClpXP (*R*) showed positive correlated
296 fluctuation, which reached the maximum when the sum of two protein production rates
297 approached to the ClpXP's processing capacity (Cookson et al., 2011; Mather et al.,
298 2010). Another theoretical analysis showed that in translation process, fluctuations of
299 mRNA-ribosome complexes (T_1^C and T_2^C) were negatively correlated (Mather et al.,
300 2013). In summary, competition can introduce negatively correlated fluctuation
301 between free targets and positively correlated fluctuation between complexes, and both
302 of their strength reach the maximum in the "*R* near-equimolar" regime.

303

304 **Regulator allocation to multiple targets**

305 Regulators often bind more than two target species simultaneously. How will regulator
306 be allocated to multiple target species? A system with multiple targets competing for
307 the same regulator can be described by the set of allocation equations (Figure 4A),
308 where the proportion of the regulator occupied by a certain target in steady state is
309 mainly determined by this target's abundance and its capabilities to bind to (and hence
310 to consume) the regulator (SI Material and Methods). It was noticed that, the form of
311 the regulator allocation equation is analogous to Kirchhoff's laws in current divider
312 circuits, where R's production rate is analogous to the total current, the capability of T_i^{C}
313 to consume R is analogous to the ith branch current, and the capability of T_i^{F} to occupy
314 R is analogous to the ith branch conductance (the reciprocal of resistance) (Figure 4B).
315 Therefore, electronic circuits and biological systems with competition may exhibit
316 similar properties, such as the "negative linear dependence" behavior when resources
317 are insufficient (in the "R scarce" regime) (Carbonell-Ballestero et al., 2016).
318 Such allocation equations have displayed in diverse mathematical models, such as
319 the reaction rates of product formation in enzymatic reactions when multiple substrates
320 competing for the same catalytic enzyme under the Michaelis-Menten kinetics (Chou
321 & Talaly, 1977), and the probabilities of promoter-TF binding when multiple promoters
322 competing for the same TF under the thermodynamic model (Bintu et al., 2005).
323 Meanwhile, this property has helped quantify the allocations of the transcription or the
324 translation resources for synthetic gene circuits (Carbonell-Ballestero et al., 2016; Qian
325 et al., 2017). We also applied such property to predict the miRNA occupancy on each

326 target site in a specific cell type with the miRNA and the target RNA expression levels,
327 and significantly improved the accuracy of the miRNA target prediction (Xie et al.,
328 2014). Those miRNAs with significant occupancy changes during tumorigenesis could
329 serve as potent biomarkers in addition to differentially expressed miRNAs.
330
331 **Discussion**
332 Competition for limited resources is ubiquitous throughout diverse molecular reactions
333 in both natural and synthetic biological systems. Using a coarse-gained mathematical
334 model, we systematically analyzed the steady-state behavior and the dynamic
335 properties of various competition network motifs, from the view of indirect regulations
336 among the competitors as well as the effects of the competitors on the regulator-target
337 response (Table 1). It should be noticed that, most of the mentioned properties are
338 connected with the concept of the *regimes* determined by the regulator-target relative
339 abundance (Figure 2A-C): threshold behavior occurs when system transfers from the
340 "*R* abundant" to the "*R* near-equimolar" regime, and linear negative dependence
341 happens when system is in the "*R* scarce" regime; while the sensitivity of the dose-
342 response curve, the correlated fluctuation, and the noise of the target level are all
343 maximized in the "*R* near-equimolar" regime.
344 Competition motif is a common network component. It seldom functions as an
345 isolated module in real-world biological systems, but often interacts with other
346 components to form complex networks. For example, simulation analysis on ceRNA

347 regulation suggested that additional targets and regulators connected with different
348 topology could enhance or weaken the ceRNA effect (Ala et al., 2013). Theoretical
349 analysis predicted that competition for degradation enzyme could either promote or
350 suppress the robustness of biological oscillating circuit with different topological
351 structures (Rondelez, 2012). In addition, competition motif could perform a variety of
352 functions by combining with other network motifs. For example, cooperating with the
353 positive feedback motif, competition can generate the winner-take-all (WTA) behavior
354 (Kim et al., 2004), which have been applied to design *in vitro* molecular circuits for
355 supervised learning and pattern classification using DNA strand displacement (Genot
356 et al., 2013; Lakin & Stefanovic, 2016).

357 The unified competition model gives inspirations for transferring knowledge
358 among different molecular scenarios, since similar molecular network topology may
359 perform similar functions. For example, the case that ceRNA competition can sharpen
360 the dose-response curve of miRNA regulation (Yuan et al., 2015) is quite similar to that
361 observed for TF titration effect (Brewster et al., 2014). Such generality and feasibility
362 give us confidence to make new predictions based on the competition model. For
363 instance, the properties of pH buffer solutions demonstrated that some weak
364 competitors could desensitize the response of the primary target to the regulator, which
365 implies the potential role of many competitors as noise buffer. Functions of numerous
366 miRNA target sites have long been a mystery that each miRNA species in mammalian
367 cell could bind to hundreds target RNA species, but only a small portion of the targets

368 with multiple high affinity binding sites could be moderately repressed (rarely exceeds
369 2-folds). That is to say, in most cases, miRNA binding are not functioned as intensive
370 repression (Seitz, 2009). Why are there so many evolutionary conserved miRNAs and
371 potential targets if this is an inefficient regulatory mechanism? The competition model
372 provides a possible explanation that such widespread miRNA competitors with low
373 binding affinity could buffer noise and stabilize gene expression.

374 Competition effect is one of the major challenges for circuits design in synthetic
375 biology. Synthetic gene expression can lead to intracellular resource reallocation, which
376 may affect the performance of both exogenous gene circuits and host gene networks
377 simultaneously. It may change the network structure of the original designed circuits
378 by introducing a hidden layer of regulation, making it difficult to predict the whole
379 circuit's behavior based on the characteristic of each individual component. For
380 example, competition for cellular resources may reshape the response of genetic
381 activation cascades in *E. coli* (Qian et al., 2017), and multiple downstream genes
382 competing for upstream signal molecules may accentuate the "retroactivity" (Brophy
383 & Voigt, 2014). It has been found that the induction strength of the synthetic gene
384 oscillator could influence the growth rate of host cell, the expression of endogenous
385 genes, and the performance of the oscillator, such as amplification and period (Weisse
386 et al., 2015). On the other hand, interestingly, using competition effect properly to
387 rebalance synthetic circuits' relation to the host cell is emerging as an effective way to
388 refine circuits performance. For example, the robustness of the synthetic oscillator can

389 be greatly improved by introducing competing binding sites for TF LacI to sharpen
390 target gene dose response curves and suppress gene expression noise (Potvin-Trottier
391 et al., 2016). Models incorporating circuit-host competition effects can predict synthetic
392 gene behaviors better (Liao et al., 2017). Reallocating the cellular translational
393 resources by introducing the endoribonuclease MazF circuit can significantly enhance
394 exogenous enzyme expression to promote metabolite production (Venturelli et al.,
395 2017). Utilizing synthetic miRNA and its competitive binding RNA sponges, a RNA-
396 based AND gate circuit was designed for selectively triggering T cell-mediated killing
397 of cancer cells (Nissim et al., 2017).

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

404

405 **Materials and Methods**

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

408

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Conflict of Interests

The authors declare that they have no conflict of interest.

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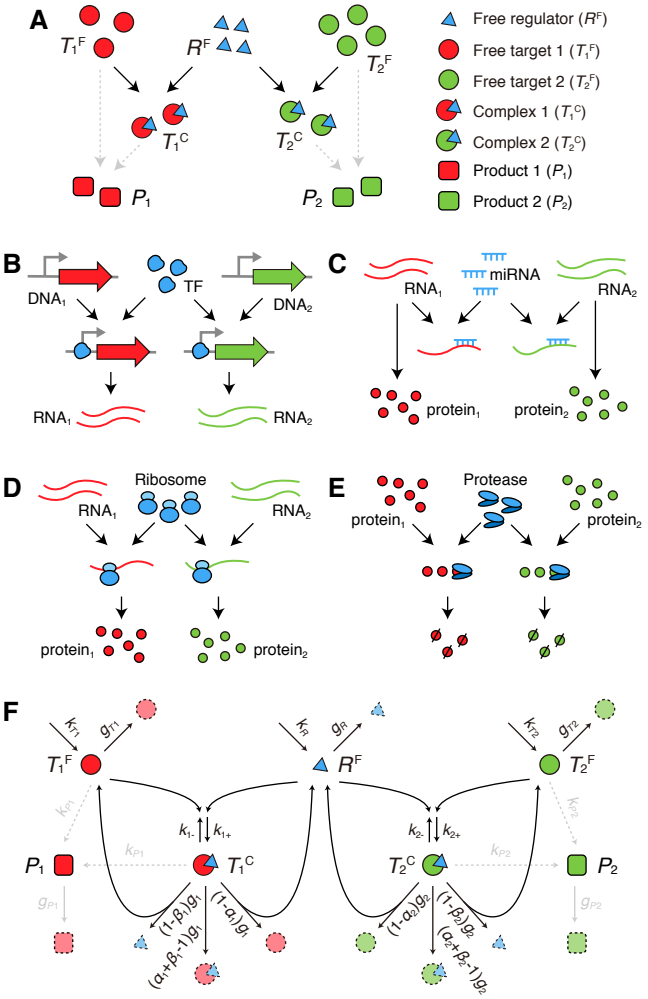

Figure 1 The coarse-gained competition motif model.

(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.

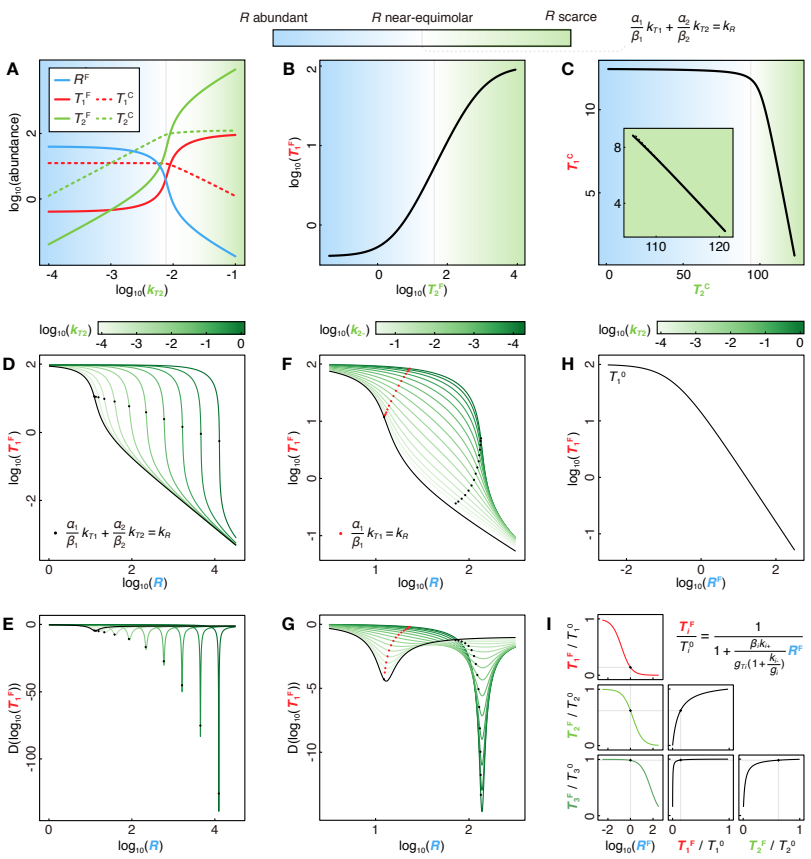

Figure 2 Steady state behaviors of competition systems.

(A-C) 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).

(D-G) Dose-response curves modulated by competition. (D-E) R-T_1^F dose-response curves (D) and their derivatives (E) with different T_2's production rate (k_{T2}). (F-G) R-T_1^F dose-response curves (F) and their derivatives (G) 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).

(H) 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.

(I) Repression folds of all targets are determined by the same R^F abundance in a multi-target repression system.

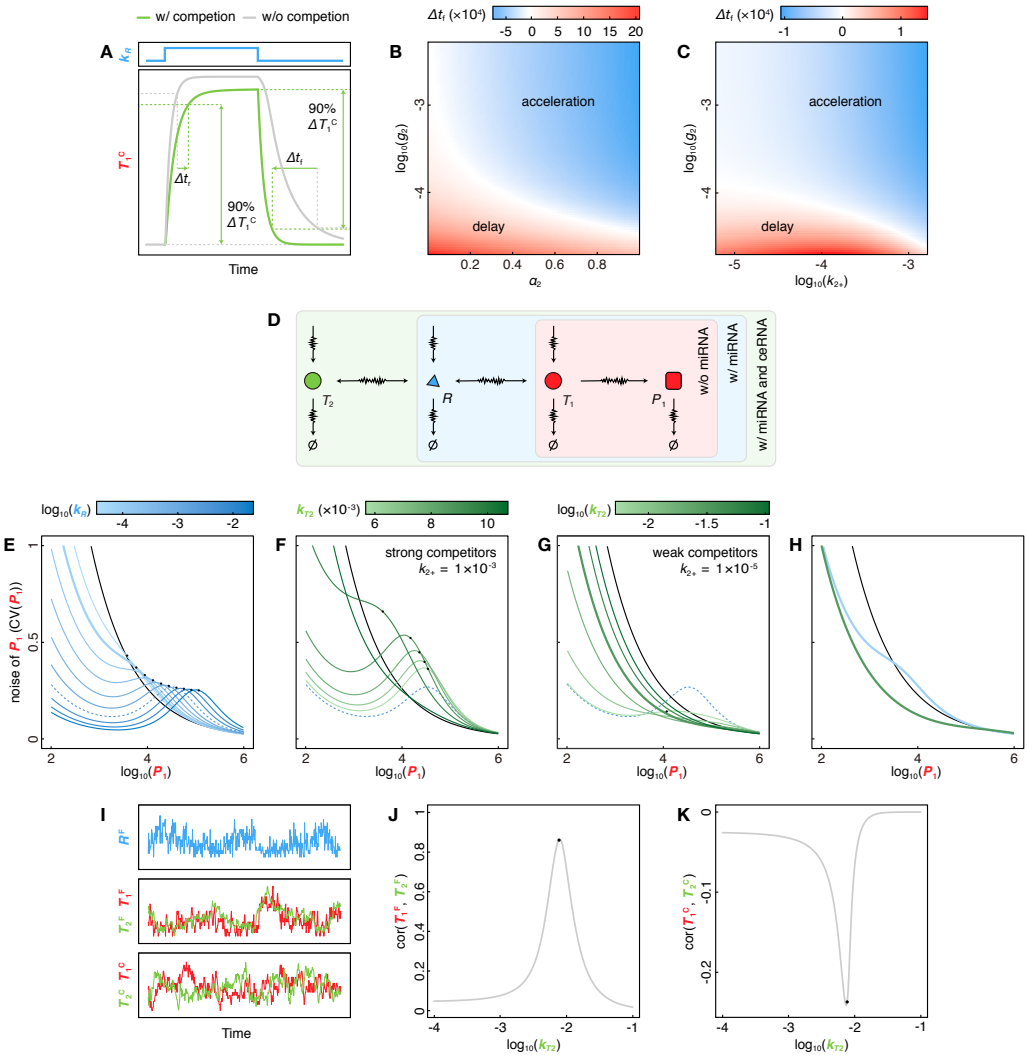

Figure 3 Dynamic properties of competition systems.

(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).

(D) Schematic diagram of the target expression noise in the miRNA-target competition scenario.

(E-H) Modification of target expression noise by competition. (E) Product expression noise (CV(P_1)) with different R's production rates (k_R). (F) CV(P_1) with different T_2's production rates (k_{T2}) where T_2 acts as a strong competitor. (G) CV(P_1) with different k_{T2} where T_2 acts as a weak competitor. (H) 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 (F) and (G). The thick blue and green lines in (H) are taken from (E) and (G) 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).

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

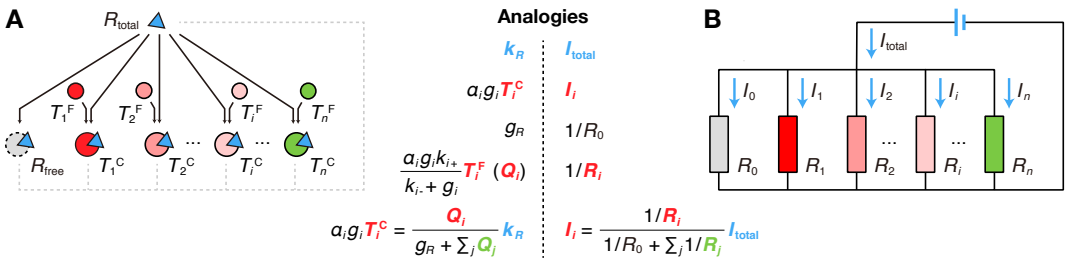

Figure 4 Regulator allocation for multi-target competition.

(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

SI Materials and Methods

1. A unified coarse-gained competition motif model.

Parameters involved in the competition motif model (Figure 1F) where two target molecule species (target#1 and #2, T_1 and T_2) competitively bind with a shared regulatory molecule species (regulator, R) are described as follows. In general, T_1, T_2 or R is produced with a rate of k_{T1}, k_{T2} or k_R, respectively. Free T_1 (T_1^{F}), T_2 (T_2^{F}) or R (R^{F}) degrades at a rate of g_{T1}, g_{T2} or g_R. T_1^{F} or T_2^{F} binds to R^{F} to form target-regulator complex T_1^{C} or T_2^{C} at a rate of k_{1+} or k_{2+}, and T_1^{C} or T_2^{C} dissociates into R^{F} and T_1^{F} or T_2^{F} at a rate of k_{1-} or k_{2-}. T_1^{C} or T_2^{C} degrades at a rate of g_1 or g_2. Regulators on the complex degrade with the possibility of α_1 or α_2, and targets on the complex degrade with the possibility of β_1 or β_2, thus regulator would recycle from T_1^{C} or T_2^{C} with the possibility of $1-\alpha_1$ or $1-\alpha_2$, target would recycle from T_1^{C} or T_2^{C} with the possibility of $1-\beta_1$ or $1-\beta_2$, and regulator and target would degrade together with the possibility of $\alpha_1+\beta_1-1$ or $\alpha_2+\beta_2-1$. When R is a repressor, T_1^{F} or T_2^{F} may generate production P_1 or P_2 at a rate of k_{P1} or k_{P2}. In contrast, when R is an activator, T_1^{C} or T_2^{C} may generate production P_1 or P_2 at a rate of k_{P1} or k_{P2}. P_1 or P_2 degrades at a rate of g_{P1} or g_{P2}.

The competing model is described in the following differential equations:

$$\frac{\mathrm{d}\,R^{\mathrm{F}}}{\mathrm{d}\,t} = k_R - g_R R^{\mathrm{F}} - (k_{1+}T_1^{\mathrm{F}} + k_{2+}T_2^{\mathrm{F}})R^{\mathrm{F}} + k_{1-}T_1^{\mathrm{C}} + k_{2-}T_2^{\mathrm{C}} + (1-\alpha_1)g_1T_1^{\mathrm{C}} + (1-\alpha_2)g_2T_2^{\mathrm{C}} \quad [1]$$

$$\frac{\mathrm{d}\,T_1^{\mathrm{F}}}{\mathrm{d}\,t} = k_{T1} - g_{T1}T_1^{\mathrm{F}} - k_{1+}T_1^{\mathrm{F}}R^{\mathrm{F}} + k_{1-}T_1^{\mathrm{C}} + (1-\beta_1)g_1T_1^{\mathrm{C}} \quad [2]$$

$$\frac{\mathrm{d}\,T_1^{\mathrm{C}}}{\mathrm{d}\,t} = k_{1+}T_1^{\mathrm{F}}R^{\mathrm{F}} - k_{1-}T_1^{\mathrm{C}} - g_1T_1^{\mathrm{C}} \quad [3]$$

$$\frac{\mathrm{d}\,T_2^{\mathrm{F}}}{\mathrm{d}\,t} = k_{T2} - g_{T2}T_2^{\mathrm{F}} - k_{2+}T_2^{\mathrm{F}}R^{\mathrm{F}} + k_{2-}T_2^{\mathrm{C}} + (1-\beta_2)g_2T_2^{\mathrm{C}} \quad [4]$$

$$\frac{\mathrm{d}\,T_2^{\mathrm{C}}}{\mathrm{d}\,t} = k_{2+}T_2^{\mathrm{F}}R^{\mathrm{F}} - k_{2-}T_2^{\mathrm{C}} - g_2T_2^{\mathrm{C}} \quad [5]$$

We used this model to describe competitions in various biological processes. In the competition for TF by DNA binding sites (Figure 1B), T_1 and T_2 represent TF binding sites on DNA and R represents TF. The production and degradation rates of DNA binding sites are set to zero because they are negligible. Complexes degrade with only TF loss ($\alpha \sim 1$, $\beta \sim 0$). When g_1 or g_2 are set to zero, there is no TF loss. For TF as activator, DNA-TF complexes (T_1^{C} and T_2^{C}) can be transcribed into RNA, while for TF as repressor, free DNAs (T_1^{F} and T_2^{F}) can be transcribed (Figure S1A). In the competition for miRNA by RNA molecules (Figure 1C), T_1 and T_2 represent

two RNA molecule species and R represents miRNA. The loss of miRNA is relatively small so β is set to zero (Figure S1B) and as miRNA acts as a repressor, only free RNAs (T_1^{F} and T_2^{F}) translate into proteins. In the case of ribosome allocation (Figure 1D), where T_1 and T_2 represent two RNA molecule species and R represents ribosome, β is also set to zero (Figure S1C). In protein degradation competition (Figure 1E), where T_1 and T_2 represent two protein molecule species and R represents the protein degradation machine, β is set to zero too (Figure S1D). The topology of miRNA-target competition, ribosome-mRNA competition and protein degradation competition are identical except that components generating further production are different.

2. Theoretically analysis for molecular environment determining shapes of the regulation between competitors.

2.1. Solving steady states. Eqs. 1-5 can be solved for steady state when giving all differentials as zero. By adding Eqs. 2 and 3 , we get

$$T_1^{\mathrm{C}} = \frac{k_{T1} - T_1^{\mathrm{F}} g_{T1}}{\beta_1 g_1} \qquad [6]$$

By adding Eqs. 1, 3 and 5, we get

$$R^{\mathrm{F}} = \frac{k_R - \alpha_1 T_1^{\mathrm{C}} g_1 - \alpha_2 T_2^{\mathrm{C}} g_2}{g_R} \qquad [7]$$

Combining Eqs. 6 and 7, we get

$$R^{\mathrm{F}} = \frac{k_R - \frac{\alpha_1}{\beta_1}(k_{T1} - T_1^{\mathrm{F}} g_{T1}) - \frac{\alpha_2}{\beta_2}(k_{T2} - T_2^{\mathrm{F}} g_{T2})}{g_R} \qquad [8]$$

Substituting Eqs. 6 and 8 into Eq. 3, we get

$$(T_1^{\mathrm{F}})^2 - T_1^{\mathrm{F}}(T_1^0 - \lambda_1 - \theta_1 + \phi_{21}) - \lambda_1 T_1^0 = 0 \qquad [9]$$

Where

$$T_1^0 = \frac{k_{T1}}{g_{T1}} \qquad T_2^0 = \frac{k_{T2}}{g_{T2}} \qquad [10]$$

$$\lambda_1 = \frac{g_R}{\alpha_1 k_{1+}}(\frac{k_{1-}}{g_1} + 1) \qquad \lambda_2 = \frac{g_R}{\alpha_2 k_{2+}}(\frac{k_{2-}}{g_2} + 1) \qquad [11]$$

$$\gamma_1 = \frac{\beta_1}{\alpha_1 g_{T1}} \qquad \gamma_2 = \frac{\beta_2}{\alpha_2 g_{T2}} \qquad [12]$$

$$\theta_1 = \gamma_1 k_R \qquad \theta_2 = \gamma_2 k_R \qquad [13]$$

$$\phi_{21} = \frac{\gamma_1}{\gamma_2}(T_2^0 - T_2^{\mathrm{F}}) \qquad \phi_{12} = \frac{\gamma_2}{\gamma_1}(T_1^0 - T_1^{\mathrm{F}}) \qquad [14]$$

Parameters were lumped to represent certain physical meanings to simplify the result. T_i^0 represents the free level of target $\#i$ (T_i) without regulators. $1/\lambda_i$ is proportional to k_{i+}, and negatively correlated with k_{i-}, thus could reflect the strength of binding affinity between T_i and regulator. θ is proportional to k_R, thus could reflect the level of regulator. ϕ_{ji} exhibits the competing regulation effects by target $\#j$ upon to target $\#i$.

Eq. 9 is a quadratic equation of T_1^{F}. Thus, the steady state abundance of free targets can be expressed as

$$T_1^{\mathrm{F}} = \frac{1}{2}(T_1^0 - \lambda_1 - \theta_1 + \phi_{21} + \sqrt{(T_1^0 - \lambda_1 - \theta_1 + \phi_{21})^2 + 4\lambda_1 T_1^0}) \quad [15]$$

$$T_2^{\mathrm{F}} = \frac{1}{2}(T_2^0 - \lambda_2 - \theta_2 + \phi_{12} + \sqrt{(T_2^0 - \lambda_2 - \theta_2 + \phi_{12})^2 + 4\lambda_2 T_2^0}) \quad [16]$$

2.2. Explanations on regimes and related phenomena. Assuming that the binding between targets and regulator is very strong, λ_i becomes negligible, thus Eq. 15 can be simplified as follows:

$$T_1^{\mathrm{F}} \simeq \begin{cases} \dfrac{\lambda_1 T_1^0}{\theta_1 - T_1^0 - \frac{\gamma_1}{\gamma_2}(T_2^0 - T_2^{\mathrm{F}})} \simeq 0 & \text{, if } T_1^0 + \phi_{21} < \theta_1 \\ T_1^0 - \theta_1 + \dfrac{\gamma_1}{\gamma_2}(T_2^0 - T_2^{\mathrm{F}}) & \text{, if } T_1^0 + \phi_{21} > \theta_1 \end{cases} \quad [17]$$

Meanwhile, the steady-state abundance of T_1^{C} and T_2^{C} can be calculated from Eq. 6:

$$T_1^{\mathrm{C}} = \frac{g_{T1}}{\beta_1 g_1}(T_1^0 - T_1^{\mathrm{F}}) \qquad T_2^{\mathrm{C}} = \frac{g_{T2}}{\beta_2 g_2}(T_2^0 - T_2^{\mathrm{F}}) \quad [18]$$

and can be simplified using Eq. 17:

$$T_1^{\mathrm{C}} \simeq \begin{cases} \dfrac{g_{T1}}{g_1 \beta_1} T_1^0 & \text{, if } T_1^0 + \phi_{21} < \theta_1 \\ \dfrac{g_{T1}}{g_1 \beta_1}(\theta_1 - \dfrac{\alpha_2 g_2 \beta_1}{\alpha_1 g_{T1}} T_2^{\mathrm{C}}) & \text{, if } T_1^0 + \phi_{21} > \theta_1 \end{cases} \quad [19]$$

The turning point in Eqs. 17 and 19:

$$T_1^0 + \phi_{21} = \theta_1 \quad [20]$$

can be regarded as a threshold to distinguish regimes of the system: "R abundant" ($T_1^0 + \phi_{21} \ll \theta_1$), "$R$ equimolar" ($T_1^0 + \phi_{21} \simeq \theta_1$) and "$R$ scarce" ($T_1^0 + \phi_{21} \gg \theta_1$). Eqs. 17 and 19 explain why the relationships between competitors are piecewise (Figure 2A-C). For T_1^{F} and T_2^{F}, according to Eq. 17, in "R abundant" regime ($T_1^0 + \phi_{21} \ll \theta_1$), almost all targets bind with R, so the level of T_1^{F} approaches to zero. In the contrary, in "R scarce" regime ($T_1^0 + \phi_{21} \gg \theta_1$), T_1^{F} increases with

the increment of T_2^{F}, which is because when the production rate of T_2 raises to sequester R, T_2^{C} increases thus $T_2^0 - T_2^{\mathrm{F}}$ increases according to Eq. 18. Given the above, when the production rate of T_2 increases to switch the system from R "abundant" regime to "R scarce" regime, the abundance of T_1^{F} will exhibit a "threshold behavior" (Figure 2B).

Similarly, Eq. 19 suggested that the relationship between T_1^{C} and T_2^{C} is piecewise linear. If R is abundant ($T_1^0 + \phi_{21} \ll \theta_1$), T_1^{C} would keep substantially unchanged, while when R is scarce ($T_1^0 + \phi_{21} \gg \theta_1$), T_1^{C} would decrease linearly with the increment of T_2^{C}, thus shows "negative linear dependence" (Figure 2C).

2.3. Approximation of the regime threshold. The threshold (Eq. 20) can be approximated based on the strong binding assumption. It is equivalent to:

$$\frac{k_{T1}}{g_{T1}} + \frac{\alpha_2\beta_1 g_{T2}}{\alpha_1\beta_2 g_{T1}}\left(\frac{k_{T2}}{g_{T2}} - T_2^{\mathrm{F}}\right) = \frac{\beta_1 k_R}{\alpha_1 g_{T1}} \qquad [21]$$

From Eq. 3, we get

$$T_1^{\mathrm{F}} = \frac{k_{1-} + g_1}{R^{\mathrm{F}} k_{1+}} T_1^{\mathrm{C}} \qquad [22]$$

According to Eq. 17, before the system reaches the threshold ($T_1^0 + \phi_{21} \leq \theta_1$) in the process of increment of production of T_2, T_1^{F} is much smaller than T_1^{C} and approaches to zero, and so does T_2^{F}. Thus, the threshold point ($T_1^0 + \phi_{21} = \theta_1$) could be approximated from Eq. 21 as:

$$\frac{\alpha_1}{\beta_1} k_{T1} + \frac{\alpha_2}{\beta_2} k_{T2} = k_R \qquad [23]$$

Eq. 23 gives an approximation of the threshold position to estimate the regime of a competing system roughly.

3. Competition can shape the regulator-target response curve.

3.1. How competition shapes regulator-target response curve. According to Eq. 9, there are

$$F(k_R, T_1^{\mathrm{F}}, T_2^{\mathrm{F}}) = (T_1^{\mathrm{F}})^2 - T_1^{\mathrm{F}}(T_1^0 - \lambda_1 - \theta_1 + \phi_{21}) - \lambda_1 T_1^0 = 0 \qquad [24]$$

$$G(k_R, T_1^{\mathrm{F}}, T_2^{\mathrm{F}}) = (T_2^{\mathrm{F}})^2 - T_2^{\mathrm{F}}(T_2^0 - \lambda_2 - \theta_2 + \phi_{12}) - \lambda_2 T_2^0 = 0 \qquad [25]$$

Thus,

$$\frac{\partial T_1^{\mathrm{F}}}{\partial k_R} = \frac{\begin{vmatrix} \frac{\partial F}{\partial k_R} & \frac{\partial F}{\partial T_2^{\mathrm{F}}} \\ \frac{\partial G}{\partial k_R} & \frac{\partial G}{\partial T_2^{\mathrm{F}}} \end{vmatrix}}{\begin{vmatrix} \frac{\partial F}{\partial T_1^{\mathrm{F}}} & \frac{\partial F}{\partial T_2^{\mathrm{F}}} \\ \frac{\partial G}{\partial T_1^{\mathrm{F}}} & \frac{\partial G}{\partial T_2^{\mathrm{F}}} \end{vmatrix}} = -\frac{\gamma_1}{1 + \frac{\lambda_1 T_1^0}{\lambda_2 T_2^0}(\frac{T_2^{\mathrm{F}}}{T_1^{\mathrm{F}}})^2 + \frac{\lambda_1 T_1^0}{(T_1^{\mathrm{F}})^2}} \qquad [26]$$

$$\frac{\partial \log T_1^{\mathrm{F}}}{\partial \log k_R} = \frac{k_R}{T_1^{\mathrm{F}}}\frac{\partial T_1^{\mathrm{F}}}{\partial k_R} = -\frac{\gamma_1 k_R}{T_1^{\mathrm{F}} + \frac{\lambda_1 T_1^0}{\lambda_2 T_2^0}\frac{(T_2^{\mathrm{F}})^2}{T_1^{\mathrm{F}}} + \frac{\lambda_1 T_1^0}{T_1^{\mathrm{F}}}} \qquad [27]$$

Eq. 27 describes the derivative of regulator-target response curve (Figure 2E and 2G).

Similarly, the buffer capacity, which quantifies the ability to resist pH changes in buffer solution, can be calculated as

$$B = -\frac{\partial k_R}{\partial \log T_1^{\mathrm{F}}} = \frac{1}{\gamma_1}(T_1^{\mathrm{F}} + \frac{\lambda_1 T_1^0}{\lambda_2 T_2^0}\frac{(T_2^{\mathrm{F}})^2}{T_1^{\mathrm{F}}} + \frac{\lambda_1 T_1^0}{T_1^{\mathrm{F}}}) \qquad [28]$$

3.2. Competition in buffer solution. For any buffer solution with a weak acid (HA) and its conjugate base (A^-) or a weak base (BOH) and its conjugate acid (B^+), there are

$$\mathrm{H_2O} \rightleftharpoons \mathrm{H^+} + \mathrm{OH^-}$$

$$\mathrm{HA} \rightleftharpoons \mathrm{H^+} + \mathrm{A^-}$$

$$\mathrm{BOH} \rightleftharpoons \mathrm{B^+} + \mathrm{OH^-}$$

Here we take ammonium buffer solution ($NH_3 \cdot H_2O$ and NH_4Cl) as an example. In a aqueous solution with a mol/L $NH_3 \cdot H_2O$ and b mol/L NH_4Cl, there are

$$\mathrm{H_2O} \rightleftharpoons \mathrm{H^+} + \mathrm{OH^-}$$

$$\mathrm{NH_3 \cdot H_2O} \rightleftharpoons \mathrm{NH_4{}^+} + \mathrm{OH^-}$$

The equilibrium constants of these two reactions are

$$K_1 = \frac{[\mathrm{H^+}][\mathrm{OH^-}]}{[\mathrm{H_2O}]} \qquad [29]$$

$$K_2 = \frac{[\mathrm{NH_4{}^+}][\mathrm{OH^-}]}{[\mathrm{NH_3 \cdot H_2O}]} \qquad [30]$$

Because the concertation water of in an aqueous solution is almost invariant, the equilibrium constant of water (ion-product constant) is defined as

$$K_{\mathrm{w}} = [\mathrm{H^+}][\mathrm{OH^-}] = K_1[\mathrm{H_2O}] \simeq 10^{-14}\,\mathrm{mol \cdot L}$$

Here, we consider H^+ (T_1) and $NH_4{}^+$ (T_2) competing for OH^- (R). Thus, Eqs. 29 and 30 is equivalent to

$$K_1 = \frac{T_1^{\mathrm{F}} R^{\mathrm{F}}}{T_1^{\mathrm{C}}} \tag{31}$$

$$K_2 = \frac{T_2^{\mathrm{F}} R^{\mathrm{F}}}{T_2^{\mathrm{C}}} \tag{32}$$

Meanwhile, because there are no production and degradation of any component, every substance is conserved as

$$T_1^{\mathrm{F}} + T_1^{\mathrm{C}} = T_1^{\mathrm{A}} = w \tag{33}$$

$$T_2^{\mathrm{F}} + T_2^{\mathrm{C}} = T_2^{\mathrm{A}} = a + b \tag{34}$$

$$T_1^{\mathrm{C}} + T_2^{\mathrm{C}} + R^{\mathrm{F}} = R^{\mathrm{A}} = a + w \tag{35}$$

Combining Eqs. 33-35, we get

$$R^{\mathrm{F}} = R^{\mathrm{A}} - T_1^{\mathrm{A}} - T_2^{\mathrm{A}} + T_1^{\mathrm{F}} + T_2^{\mathrm{F}} \tag{36}$$

Combining Eqs. 31, 32 and 36, we get

$$T_1^{\mathrm{F}} = \frac{1}{2}(T_1^{\mathrm{A}} + T_2^{\mathrm{A}} - R^{\mathrm{A}} - T_2^{\mathrm{F}} - K_1 + \sqrt{(T_1^{\mathrm{A}} + T_2^{\mathrm{A}} - R^{\mathrm{A}} - T_2^{\mathrm{F}} - K_1)^2 + 4K_1T_1^0}) \tag{37}$$

which is a degenerate form of Eq. 15, where

$$\alpha_1 = \alpha_2 = \beta_1 = \beta_2 \qquad g_{T1} = g_{T2}$$

$$K_1 = \frac{g_R}{\alpha_1 k_{1+}}(\frac{k_{1-}}{g_1} + 1) \qquad K_2 = \frac{g_R}{\alpha_2 k_{2+}}(\frac{k_{2-}}{g_2} + 1)$$

$$T_1^{\mathrm{A}} = k_{T1}/g_{T1} \qquad T_2^{\mathrm{A}} = k_{T2}/g_{T2}$$

$$R^{\mathrm{A}} = k_R/g_{T1} = k_R/g_{T2}$$

According to Eq. 28, the buffer capacity of this solution is

$$B = \frac{\partial R}{\partial \mathrm{pOH}} = T_1^{\mathrm{F}} + \frac{K_{\mathrm{w}}}{K_2 T_2^{\mathrm{A}}}\frac{(T_2^{\mathrm{F}})^2}{T_1^{\mathrm{F}}} + \frac{K_{\mathrm{w}}}{T_1^{\mathrm{F}}} = [\mathrm{OH}^-] + \frac{K_2(a+b)[\mathrm{OH}^-]}{(K_2 + [\mathrm{OH}^-])^2} + [\mathrm{H}^+] \tag{38}$$

Eq. 38 indicates that when a mild change of OH^- is introduced to the solution, the buffer capacity guarantees the stable of pOH (and pH). More buffer substance ($NH_4{}^+$ and $NH_3 \cdot H_2O$, $a + b$) can lead to a larger buffer capacity, and the buffer capacity may maximize when $\mathrm{pH} = \mathrm{p}K_2$.

3.3. Dose-response curve of free target to free regulator. Substituting Eq. 22 into Eq. 2, we get

$$k_{T1} - T_1^{\mathrm{F}} g_{T1} - k_{1+} R^{\mathrm{F}} T_1^{\mathrm{F}} + (k_{1-} + (1-\beta_1) g_1) T_1^{\mathrm{C}} = 0 \qquad [39]$$

Thus,

$$\frac{T_1^{\mathrm{F}}}{T_1^0} = \frac{1}{1 + O_1 R^{\mathrm{F}}}, \text{ where } O_1 = \frac{\beta_1 k_{1+}}{g_{T1}(1 + \frac{k_{1-}}{g_1})} \qquad [40]$$

In system with n targets competing for same regulator, for the ith target ($i = 1,\ 2,\ \cdots,\ n$), this result can be extended as:

$$\frac{T_i^{\mathrm{F}}}{T_i^0} = \frac{1}{1 + O_i R^{\mathrm{F}}}, \text{ where } O_i = \frac{\beta_i k_{i+}}{g_{Ti}(1 + \frac{k_{i-}}{g_i})} \qquad [41]$$

Similarly,

$$\frac{T_i^{\mathrm{C}}}{T_i^0} = \frac{g_{Ti}}{\beta_i g_i}(1 - \frac{1}{1 + O_i R^{\mathrm{F}}}) \qquad [42]$$

Eqs. 41 and 42 indicate that the level of T_i^{F} and T_i^{C} are determined only by the free level of R, and some chemical kinetic parameters of T_i and R. In another wordsif two or more tagets compete for shared R, the relative abundances of each free target or complex are independent of other targets when giving the free level of R. In the siRNA design strategy (Yuan et al., 2015; Yuan et al., 2016),this property guarantees that no matter what expression of the off-target gene is (unless the expression is zero), the amount of free siRNA required to repress the target gene to a certain extent would always repress the off-target gene to a certain extent, which is determined by O_{on} and O_{off}, as described in Eq. 41. When giving the expression of any target gene, siRNA could act as a medium to predict the expression of other target genes. This property also guides how to select suitable chemical reaction parameters: a good siRNA should have large O_{on} and small O_{off}.

4. Competition can delay or accelerate dynamic response.

When R level changes, comparisons of $\frac{\mathrm{d}\,R^{\mathrm{F}}}{\mathrm{d}\,t}$ tells how competition affects the dynamic response speed of T_1 with respect to R. According to Eq. 1 and 5,

$$\begin{aligned}\frac{\mathrm{d}\,R^{\mathrm{F}}}{\mathrm{d}\,t} =& k_R - g_R R^{\mathrm{F}} - k_{1+} T_1^{\mathrm{F}} R^{\mathrm{F}} + k_{1-} T_1^{\mathrm{C}} + (1-\alpha_1) g_1 T_1^{\mathrm{C}} \\ & \underbrace{-k_{2+} T_2^{\mathrm{F}} R^{\mathrm{F}} + k_{2-} T_2^{\mathrm{C}} + g_2 T_2^{\mathrm{C}}}_{\mathrm{A}} \ \underbrace{-\alpha_2 g_2 T_2^{\mathrm{C}}}_{\mathrm{B}}\end{aligned} \qquad [43]$$

Item A equals to $-\frac{\mathrm{d}\,T_2^{\mathrm{C}}}{\mathrm{d}\,t}$, and indicates the ability of T_2 to sequester R when T_2^{C} forms ($-\frac{\mathrm{d}\,T_2^{\mathrm{C}}}{\mathrm{d}\,t} < 0$), or release R when T_2^{C} dissociates ($-\frac{\mathrm{d}\,T_2^{\mathrm{C}}}{\mathrm{d}\,t} > 0$). Item B indicates the level of R loss mediated by T_2^{C} degradation.

On the rising edge of R, T_2^{C} forms so item A < 0, meanwhile item B < 0 all the time, thus $\frac{\mathrm{d}R^{\mathrm{F}}}{\mathrm{d}t}$ is smaller than non-competing system, leading to a slower response. On the falling edge of R, item A > 0 while item B < 0, thus the response speed depends on the relative magnitude of item A and B. As g_2 or α_2 increases, the absolute value of item B increases to alter the response from delay to acceleration. As k_2^+ increases or k_2^- decreases, item A increases while the absolute value of item B also increases because T_2^{C} becomes larger, thus delay the response when there is no R loss mediated by T_2^{C} ($g_2 = 0$, Figure S3D), alter the response from delay to acceleration when g_2 is moderate (Figure S3E), or accelerate the response when g_2 is large enough (Figure S3F).

5. Noise and correlated flucuation evaluation.

The variances and co-variances of the molecular species in the system can be estimated with linear noise approximation. Fluctuation-dissipation theorem provides a general way to quantifies the fluctuations. The fluctuation-dissipation equation was solved numerically to calculate the covariance matrix $\boldsymbol{C}$, the diagonal elements of which are the variance of corresponding entities, while the off-diagonal elements of which describe the co-variances between molecular species. The noise of a molecular species i is defined as coefficient of variation $\sigma(x_i)/\bar{x}_i$ and the correlation between two molecular species i and j is defined as $\mathrm{cov}(x, y)/(\sigma(x_i)\sigma(x_j))$, where x_i and x_j are random variables representing the abundance of molecular species i and j.

Taking miRNA competing system as an example (where miRNA acts as repressor), the vector of molecular number

$$\boldsymbol{N} = \begin{bmatrix} R^{\mathrm{F}} & T_1^{\mathrm{F}} & T_2^{\mathrm{F}} & T_1^{\mathrm{C}} & T_2^{\mathrm{C}} & P_1 & P_2 \end{bmatrix} \tag{44}$$

Transition rates vector is

$$\begin{aligned} \boldsymbol{f}(\boldsymbol{x}) = \Big[\; & k_{T1} \quad g_{T1}T_1^{\mathrm{F}} \quad k_{1+}T_1^{\mathrm{F}}R^{\mathrm{F}} \quad k_{1-}T_1^{\mathrm{C}} \quad g_1\left(1-\beta_1\right)T_1^{\mathrm{C}} \quad k_{T2} \quad g_{T2}T_2^{\mathrm{F}} \\ & k_{2+}T_2^{\mathrm{F}}R^{\mathrm{F}} \quad k_{2-}T_2^{\mathrm{C}} \quad g_2\left(1-\beta_2\right)T_2^{\mathrm{C}} \quad k_R \quad g_R R^{\mathrm{F}} \quad g_1\left(1-\alpha_1\right)T_1^{\mathrm{C}} \\ & g_1\left(\alpha_1+\beta_1-1\right)T_1^{\mathrm{C}} \quad g_2\left(1-\alpha_2\right)T_2^{\mathrm{C}} \quad g_2\left(\alpha_2+\beta_2-1\right)T_2^{\mathrm{C}} \quad k_{P1}T_1^{\mathrm{F}} \\ & g_{P1}P_1 \quad k_{P2}T_2^{\mathrm{F}} \quad g_{P2}P_2 \Big] \end{aligned} \tag{45}$$

Stoichiometric matrix is

$$\boldsymbol{S} = \begin{bmatrix} 1 & -1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 & 0 & -1 & 1 & 0 & 1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \quad [46]$$

In the steady state, the rate equations can be linearized by the Jacobian matrix:

$$\boldsymbol{J} = \boldsymbol{S} \cdot \frac{\partial \boldsymbol{f}(\boldsymbol{x})}{\partial \boldsymbol{N}} \quad [47]$$

The diffusion matrix $\boldsymbol{D}$ is

$$\boldsymbol{D} = \boldsymbol{S} \cdot \mathrm{diag}\left(\boldsymbol{f}(\boldsymbol{x})\right) \cdot \boldsymbol{S}^{\top} \quad [48]$$

Therefore, the covariance matrix $\boldsymbol{C}$ can be calculate numerically by solving the fluctuation dissipation equation:

$$\boldsymbol{J} \cdot \boldsymbol{C} + \boldsymbol{C} \cdot \boldsymbol{J}^{\top} + \boldsymbol{D} = \boldsymbol{0} \quad [49]$$

6. Regulator allocation to multiple targets.

When there are n targets, similarly to Eqs. 1-5, there are

$$\frac{\mathrm{d}\,R^{\mathrm{F}}}{\mathrm{d}\,t} = k_R - g_R R^{\mathrm{F}} + \sum_{i=1}^{n}(-k_{i+}T_i^{\mathrm{F}}R^{\mathrm{F}} + k_{i-}T_i^{\mathrm{C}} + (1-\alpha_i)g_i T_i^{\mathrm{C}}) \quad [50]$$

$$\frac{\mathrm{d}\,T_i^{\mathrm{F}}}{\mathrm{d}\,t} = k_{Ti} - g_{Ti}T_i^{\mathrm{F}} - k_{i+}T_i^{\mathrm{F}}R^{\mathrm{F}} + k_{i-}T_i^{\mathrm{C}} + (1-\beta_i)g_i T_i^{\mathrm{C}} \quad [51]$$

$$\frac{\mathrm{d}\,T_i^{\mathrm{C}}}{\mathrm{d}\,t} = k_{i+}T_i^{\mathrm{F}}R^{\mathrm{F}} - k_{i-}T_i^{\mathrm{C}} - g_i T_i^{\mathrm{C}} \quad [52]$$

At steady states, by adding Eqs. 50 and 52, we get

$$R^{\mathrm{F}} = \frac{k_R - \sum_i \alpha_i T_i^{\mathrm{C}} g_i}{g_R} \quad [53]$$

Solving Eq. 52, we get

$$T_i^{\mathrm{F}} = \frac{k_{i-} + g_i}{R^{\mathrm{F}} k_{i+}} T_i^{\mathrm{C}} \quad [54]$$

Combining Eqs. 53 and 54, we get

$$R^{\mathrm{F}} = \frac{k_R}{g_R + \sum_i Q_i} \quad [55]$$

where

$$Q_i = \alpha_i g_i \frac{k_{i+}}{k_{i-} + g_i} T_i^{\mathrm{F}} \quad [56]$$

Thus,

$$\alpha_i g_i T_i^{\mathrm{C}} = \frac{Q_i}{g_R + \sum_j Q_j} k_R \quad [57]$$

Eq. 57 has the exact form of current divider rule in electronics:

$$I_i = \frac{\frac{1}{R_i}}{\frac{1}{R_0} + \sum_j \frac{1}{R_j}} I_{\mathrm{total}} \quad [58]$$

It inspires that R's production rate (k_R) is analogous to the total current (I_{total}); the capability of T_i^{C} to consume R ($\alpha_i g_i T_i^C$) is analogous to the ith branch current (I_i); and the capability of T_i^{F} to occupy R (Q_i) is analogous to the ith branch conductance ($1/R_i$).

When R is scarce, $T_i^{\mathrm{F}} \simeq T_i^0$, thus Eq. 56 is approximated to

$$Q_i \simeq \alpha_i g_i \frac{k_{i+}}{k_{i-} + g_i} T_i^0 \quad [59]$$

which indicates that in the "R scarce" regime, the capability of T_i to occupy R (resistance) is only determined by the parameter settings of T_i.

For catalytic reactions with a constat level of enzyme (regulator) and substances (targets), Eqs. 50-52 degenerate as

$$\frac{\mathrm{d}\, R^{\mathrm{F}}}{\mathrm{d}\, t} = \sum_{i=1}^{n} (-k_{i+} T_i^{\mathrm{F}} R^{\mathrm{F}} + k_{i-} T_i^{\mathrm{C}} + g_i T_i^{\mathrm{C}}) \quad [60]$$

$$\frac{\mathrm{d}\, T_i^{\mathrm{F}}}{\mathrm{d}\, t} = -k_{i+} T_i^{\mathrm{F}} R^{\mathrm{F}} + k_{i-} T_i^{\mathrm{C}} \quad [61]$$

$$\frac{\mathrm{d}\, T_i^{\mathrm{C}}}{\mathrm{d}\, t} = k_{i+} T_i^{\mathrm{F}} R^{\mathrm{F}} - k_{i-} T_i^{\mathrm{C}} - g_i T_i^{\mathrm{C}} \quad [62]$$

Under the assumption of Michaelis-Menten kinetics that $\frac{\mathrm{d}\, T_i^{\mathrm{C}}}{\mathrm{d}\, t} = 0$, define $K_i = (k_{i-} + g_i)/k_{i+}$, then we get

$$T_i^{\mathrm{C}} = \frac{T_i^{\mathrm{F}}}{K_i} R^{\mathrm{F}} \quad [63]$$

Thus,

$$R^{\mathrm{total}} = R^{\mathrm{F}} + \sum_j T_j^{\mathrm{C}} = R^{\mathrm{F}} (1 + \sum_j T_j^{\mathrm{F}} / K_j) \quad [64]$$

$$R^{\mathrm{F}} = \frac{1}{1 + \sum_j T_i^{\mathrm{F}} / K_i} R^{\mathrm{total}} \quad [65]$$

$$T_i^{\mathrm{C}} = \frac{T_i^{\mathrm{F}} / K_i}{1 + \sum_j T_j^{\mathrm{F}} / K_j} R^{\mathrm{total}} \quad [66]$$

Which is the formation of enzyme allocation in Michaelis-Menten kinetics systems (Chou and Talaly, 1977).

7. Simulation parameters for drawing figures.

The scales of simulation parameters are referenced from previous publications across different competition scenarios, such as transcription (Jayanthi et al., 2013), post-transcription (Ala et al., 2013; Schmiedel et al., 2015),translation (Gorochowski et al., 2016),degradation (Cookson et al., 2011)and chemical buffer solutions. Table S1 lists the parameters for drawing figures, the scales of which are derived from from previous researches on ceRNA effects (Ala et al., 2013; Yuan et al., 2015; Yuan et al., 2016)All gradually changing parameters are shown in figures. In Figure 2F-G and S2D-E, $k_{T2} = 1 \times 10^{-2}$. In Figure 2I, $k_{T2} = 1 \times 10^{-4}$, and parameters of T_3 are shown in Table S1. In Figure 3A-C and S3A-F, $g_1 = 4 \times 10^{-5}$, $k_{T2} = 1 \times 10^{-4}$. In Figure S3A, $g_2 = 3.2 \times 10^{-4}$. In Figure 3E-H, for dashed blue lines, $k_R = 5 \times 10^{-3}$; for thick blue lines, $k_R = 7.81 \times 10^{-5}$; for thick green lines, $k_R = 5 \times 10^{-3}$, $k_{T2} = 3.21 \times 10^{-2}$. In Figure S3G-J, $k_{T1} = 5 \times 10^{-3}$.

Table S1. Primary parameters for simulations

Primary parameters						Additional parameters for Figure 2I	
R		T_1		T_2		T_3	
k_R	5×10^{-3}	k_{T1}	1×10^{-3}	k_{T2}	8×10^{-3}	k_{T3}	5×10^{-4}
g_R	1×10^{-4}	g_{T1}	1×10^{-5}	g_{T2}	1×10^{-5}	g_{T3}	1×10^{-5}
		k_{1+}	1×10^{-4}	k_{2+}	1×10^{-4}	k_{3+}	1×10^{-6}
		k_{1-}	5×10^{-5}	k_{2-}	5×10^{-5}	k_{3-}	5×10^{-5}
		g_1	8×10^{-5}	g_2	8×10^{-5}	g_3	1×10^{-5}
		α_1	1	α_2	0.5	α_3	0.5
		β_1	1	β_2	1	β_3	1
		k_{P1}	1×10^{-2}				
		g_{P1}	5×10^{-6}				

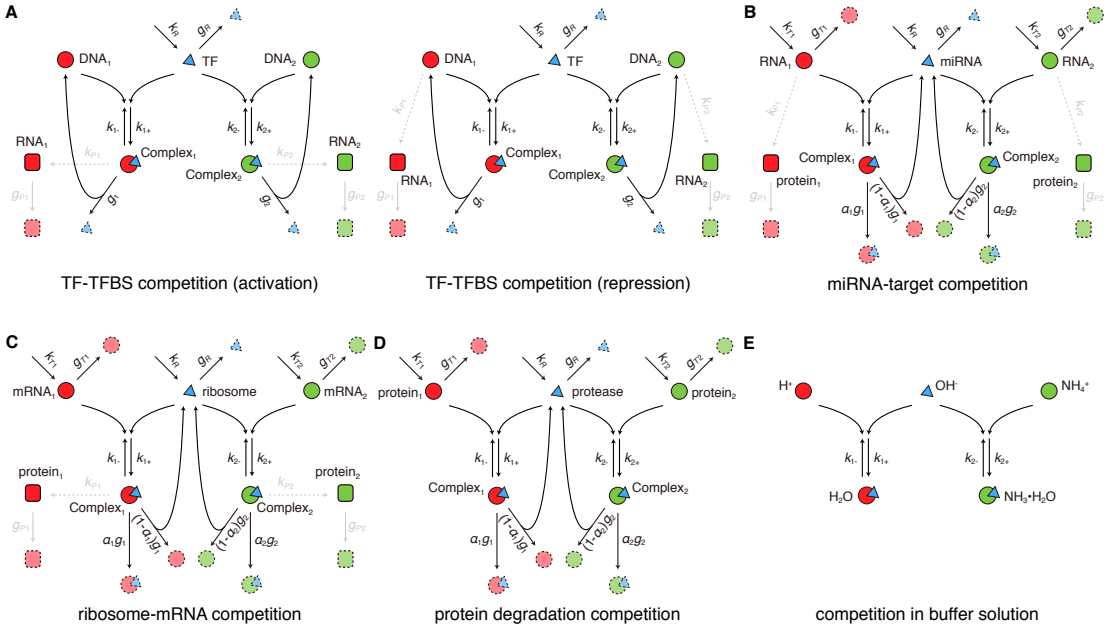

Figure S1 Detailed descriptions under the unified coarse-gained competition motif model for diverse competition scenarios.

(A) DNA TF binding sites (TFBS) competing for TFs. Left: TF acts as an activator; right: TF acts as a repressor.

(B) RNA molecules competing for miRNAs.

(C) RNA molecules competing for ribosomes.

(D) Proteins competing for proteases.

(E) Competition in the ammonium buffer solution, where H^+ and NH_4^+ compete for OH^-.

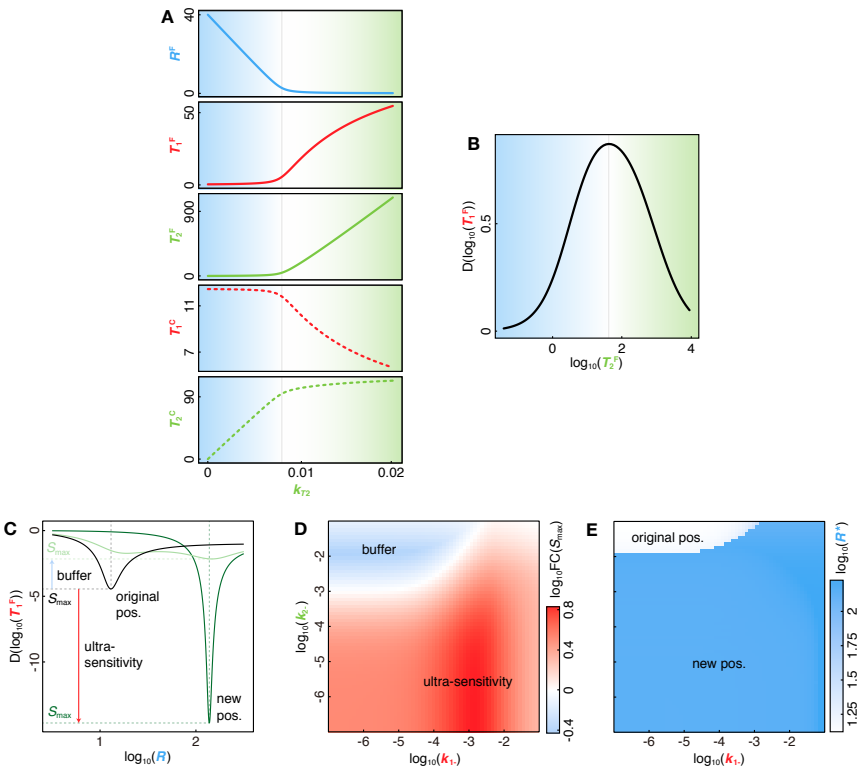

Figure S2 Steady state behaviors of competition systems.

(A) Abundances of each component in Figure 2A on linear scales.

(B) Derivatives of the curve in Figure 2B. Each component's abundance in competition motif changing with T_2's production rate (k_{T2}). Colors, lines and parameter settings are the same with Figure 2A-C.

(C) Schematic diagram depicting the maximum sensitivity (S_{max}) and its position (R^*) of R-T_1 dose-response curves. Dose-response curves are adopted from Figure 3G.

(D-E) Relative binding affinities of T_1 and T_2 (k_{1-} and k_{2-}) shape R-T_1 dose-response curves. (D) Fold change of S_{max} compared with that of non-competing system (k_{T2}=0). Competition of T_2 buffers the response of T_1^F to R when $\log_{10}FC(S_{max})<0$, but introduces larger sensitivity when $\log_{10}FC(S_{max})>0$. (E) Changes of R^*.

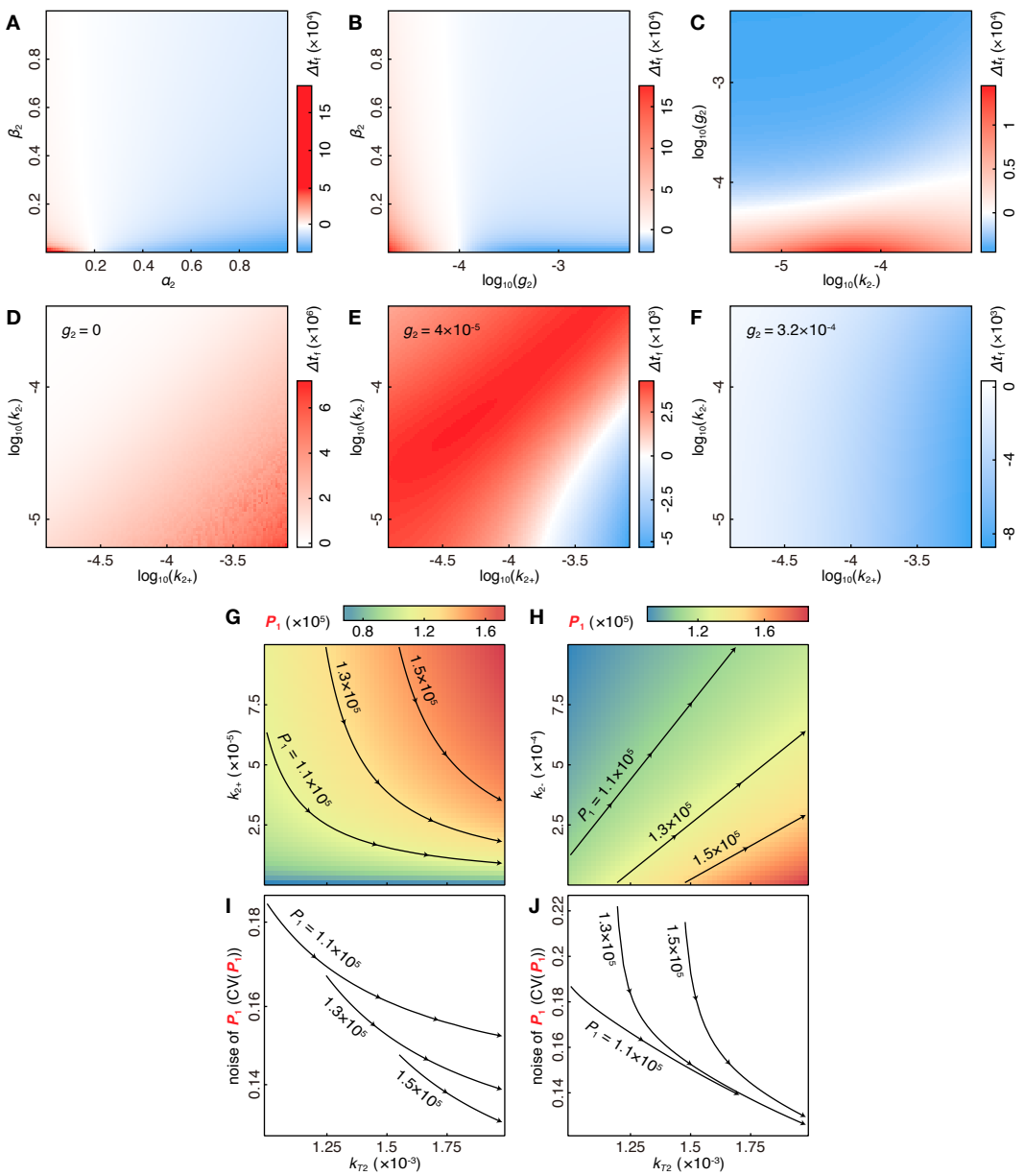

Figure S3 Dynamic properties of competition systems.

(A-F) Modifications of response time on the falling edge of R's change under different kinetic parameters: (A) different α_2 and β_2; (B) different g_2 and β_2; (C) different k_{2-} and g_2; (D-F) different k_{2+}, k_{2-} and g_2 (values of g_2 are shown in figures).

(G-J) Abundant weak competitors can buffer target expression noise better. (G-H) P_1 level changing with T_2's production rate (k_{T2}) and T_2's association rate (k_{2+}) (G), or k_{T2} and T_2's dissociation rate (k_{2-}) (H). Black lines are T_1^F level isolines. (I-J) Target expression noise changes on the isolines in (G) and (H) respectively. Along the direction of the arrows, T_2's production increases and T_2's binding affinity decreases, bringing about lower expression noise.