Origin of co-operativity in the activation of dimeric transcription factors

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Cooperative behavior in the binding of ligands to a protein is often viewed as a complex phenomenon where conformational changes induced by the binding of the first ligand leads to tighter binding of subsequent ligands. We revisit the ligand-dependent activation of dimeric transcription factors and show that this process may appear cooperative even when it results from independent ligand binding events. This effect is further accentuated through binding of the activated transcription factor to its cognate operator site on the DNA, where we demonstrate that cooperative activation is a stable fixed point. Our analysis nicely accounts for the apparent co-operativity inherent in the biological activity of many dimeric transcription factors.

I. INTRODUCTION

Intracellular sensing of the concentration of a signal molecule may depend on the binding of the ligand to a regulator protein, which, in turn, controls transcription from specific promoters. Many of these transcriptional regulators (also known as transcription factors (TFs)) are known to function as dimers, capable of recognizing discrete DNA sequence motifs in the regulatory regions (operators) associated with the cognate promoter(s). One may therefore express the activity of the TF by the occupancy of the operator as a function of signal molecule (ligand) concentration. Below, we will revisit the process of forming active TF dimers as a consequence of ligand binding to the protein and subsequent binding of the activated TF-ligand complex to the DNA. Our analysis was inspired by a bacterial cell-cell communication mechanism called "quorum sensing". In quorum sensing, a freely-diffusible self-produced signal molecule (frequently, an N-acylated homoserine lactone or AHL) accumulates in the culture. Once the AHL concentration exceeds a critical threshold concentration (thought to be determined primarily by binding of the AHL to a LuxR-type TF), the TF becomes activated and elicits the transcription of key target genes, which are often involved in bacterial virulence [1, 2]. The resulting phenotypic "switch" is often very abrupt, and has all the hallmarks of being underpinned by a highly cooperative mechanism [3–5]. The modeling of these quorum sensing systems has been challenged by the controversy as to whether the LuxR-type TFs associated with quorum sensing are of the common form where TF dimerization drives ligand binding, or whether some are of the form where ligand binding drives TF dimerization [6–9]. The controversy was partially resolved when Sappington and coworkers [10] demonstrated that, *in vivo*, LasR folds reversibly into its active form prior to reversibly binding ligands. This is consistent with the kinetic study reported by Claussen *et al.* [11]. We shall, therefore, take the "normal" case where dimerization of TFs precedes ligand bindings as our starting point and leave the formalism for the case where ligand binding precedes dimerization to an appendix. While looking into the details of the TF activation, we noted that driving of the system by continuous production and turnover of the TF leads to a rescaled dissociation constant and increased cooperativity. In the current work, we derive an expression for the effective dissociation constant of the ligand-TF complex, and establish a proper independence measure for the ligand bindings. The formalism clarifies how it is possible to discriminate between cooperative and non-cooperative activation, and between fast and slow response to changes in ligand concentration, depending on the rate of TF turnover in monomer, dimer, and ligand-bound forms.

The form $s^h/(K_d^h + s^h)$, where s is the ligand concentration, K_d is the dissociation constant, and h is the "cooperativity coefficient" (also known as the Hill coefficient), was proposed by Hill in his analysis of the oxygen transport properties of haemoglobin. This expression describes receptor occupancy well at ligand concentrations approximating to the K_d [12–14]. However, the Hill expression fails to describe the dynamic range of an activated regulator, such as a TF well. In the current work, we remedy this by showing that the concentration of the activated dimer can be approximated by the expression;

$$r_a = \frac{s^2}{K_e^2 + 2\zeta_e K_e s + s^2}\, r_m \tag{1}$$

$$= \begin{cases} \dfrac{s^2}{K_e^2 + s^2}\, r_m & ,\ \zeta_e = 0 \text{ (cooperative)} \\ \dfrac{s^2}{(K_e + s)^2}\, r_m & ,\ \zeta_e = 1 \text{ (indepentent)} \end{cases} \tag{2}$$

where r_m denotes the saturated concentration of the activated form of the transcription factor, K_e denotes the effective dissociation constant (which may differ from the underlying dissociation constant for ligand binding to the dimeric regulator), and where ζ_e is the independence measure, which lies between 0 (cooperative ligand

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bindings) and 1 (a product of two independent ligand bindings).

II. ANALYSIS

Many TFs function as homo- or heterodimers. Furthermore, they play an important role as a building block in bi-stable biological switches [15]. We shall primarily be concerned with the typical case where homodimerization drives ligand binding. However, for completeness, a brief account of the case where ligand binding precedes dimerization is presented in the appendix.

A. Dimerization drives ligand binding

The kinetics describing constitutive production of the transcription factor (R), the dimerization of the TF, and its activation trough ligand (S) binding are;

$$\xrightarrow{b_1} \mathrm{R} \qquad \mathrm{R} \xrightarrow{\lambda_1} \tag{3}$$

$$2\mathrm{R} \overset{k_2^{\pm}}{\rightleftarrows} \mathrm{R}_2 \qquad \mathrm{R}_2 \xrightarrow{\lambda_2} \tag{4}$$

$$\mathrm{R}_2 + \mathrm{S} \overset{k_3^{\pm}}{\rightleftarrows} \mathrm{R}_2\mathrm{S} \qquad \mathrm{R}_2\mathrm{S} \xrightarrow{\lambda_3} \tag{5}$$

$$\mathrm{R}_2\mathrm{S} + \mathrm{S} \overset{k_4^{\pm}}{\rightleftarrows} \mathrm{R}_2\mathrm{S}_2 \qquad \mathrm{R}_2\mathrm{S}_2 \xrightarrow{\lambda_4} \tag{6}$$

as illustrated in Fig. 1. Here, the constitutive production of TFs, b_1, encompasses mRNA transcription as well as translation into TFs. On-rate constants for the dimerization and ligand bindings are superscripted "+" and off-rates are superscripted "−". Proteolytic degradation and dilution by cell growth are both contained in the degradation rates λ_i. Note that we allow for dissociation of the dimerized TF (R_2). Should the dimer be stable, i. e. when k_2^- is negligible, the formalism still applies.

If we denote the concentrations r_1=[R], r_2=[R_2], r_3=[R_2S], r_4=r_a=[$\mathrm{R}_2\mathrm{S}_2$], and s=[S], the kinetic equations corresponding to Eqs. (3)-(6) are;

$$\frac{dr_1}{dt} = b_1 + 2k_2^- r_2 - 2k_2^+ r_1^2 - \lambda_1 r_1 \tag{7}$$

$$\frac{dr_2}{dt} = k_2^+ r_1^2 + k_3^- r_3 - 2k_3^+ r_2 s - k_2^- r_2 - \lambda_2 r_2 \tag{8}$$

$$\frac{dr_3}{dt} = 2k_3^+ r_2 s + 2k_4^- r_4 - k_4^+ r_3 s - k_3^- r_3 - \lambda_3 r_3 \tag{9}$$

$$\frac{dr_4}{dt} = k_4^+ r_3 s - 2k_4^- r_4 - \lambda_4 r_4 \tag{10}$$

The explicit inclusion of the proper statistical weights (factors of 2) allows for simpler interpretation of independence in the analysis.

The maximal activated concentration, r_m, the effective dissociation constant, K_e, and the independence measure, ζ_e, at steady state, will now be determined. To determine the effective dissociation constant (K_e) in the parametrization of the activated regulator in Eq.(1), we consider its asymptotic behavior at small and large values of s for the second order process. On a logarithmic scale, these are recognized as straight lines (of slope 2 and 0 respectively) and the effective dissociation constant sits at their crossing. For independent ligand bindings, r_a deviates a factor 4 from its maximal value at the crossing of the asymptotes, while it only deviates a factor 2 for cooperative ligand bindings. Its actual deviation at the crossing of the asymptotes will be used to determine the independence measure. The remainder of this section, concerns the mathematical details of this procedure.

When s is large, r_2 and r_3 are negligible and r_1 assumes its minimal value;

$$r_{1\min} = \frac{\lambda_1}{4k_2^+}\left(\sqrt{1 + \frac{8b_1 k_2^+}{\lambda_1^2}} - 1\right) \tag{11}$$

which can be seen by setting $dr_1/dt = 0$ with $r_2 = 0$. The maximal value of r_4 is then;

$$r_{4\max} = r_m = \frac{b_1 - \lambda_1 r_{1\min}}{2\lambda_4} \tag{12}$$

which is just a net dimer source term diluted by growth and degradation (λ_4). When s is small r_1 and r_2 will be at their maximal value, and r_4 will be dominated by the s^2 behavior. In this limit we have;

$$r_{1\max} = K_2 \frac{\lambda_1}{2\lambda_2}\frac{1}{2}\left(\sqrt{1 + \frac{8b_1\lambda_2}{\lambda_1^2 K_2}} - 1\right) \tag{13}$$

$$r_{2\max} = \frac{r_{1\max}^2}{K_2} \tag{14}$$

$$K_2 = \frac{k_2^- + \lambda_2}{k_2^+} \tag{15}$$

and, to leading order in s, we find;

$$r_4 = \frac{r_{1\max}^2}{K_2}\frac{s^2}{K_3 K_4} \tag{16}$$

$$K_3 = \frac{k_3^- + \lambda_3}{k_2^+} \approx \frac{k_3^-}{k_2^+} \tag{17}$$

$$K_4 = \frac{k_4^- + \lambda_4/2}{k_4^+} \approx \frac{k_4^-}{k_4^+} \tag{18}$$

where K_2, K_3, and K_4, are recognized as the dissociation constants for the processes (4)-(6). The asymptotes, Eq. (12) and Eq. (16), cross at the effective dissociation constant;

$$K_e^2 = \frac{r_{4\max}}{r_{2\max}} K_3 K_4 \tag{19}$$

Assuming dimer forms are equally protected against proteolytic turnover, the effective dissociation constant will be larger than the dissociation constant for the second order binding of the ligand, $(K_3K_4)^{1/2}$.

The effective independence, ζ_e, is determined by setting the parametrization, Eq. (1), equal to the steady state value of r_a at $s = K_e$. We find the independence;

$$\zeta_e = \frac{r_m}{2\,r_a} - 1 \tag{20}$$

which has the value 1 for independent ligand bindings and 0 for fully cooperative bindings.

The timescale related to the changes in regulator concentration can be isolated by summing Eqs. (7)-(10). Each of the dimeric species contains two regulator constituents and is therefore counted twice. We then arrive at;

$$\frac{dr}{dt} = b_1 - \lambda r \tag{21}$$

$$r = r_1 + 2r_2 + 2r_3 + 2r_4 \tag{22}$$

$$\lambda\, r = r_1\lambda_1 + 2r_2\lambda_2 + 2r_3\lambda_3 + 2r_4\lambda_4 \tag{23}$$

where r is the total regulator concentration and λ is the weighted average of the dilution and degradation rates. Eq. (21) governs the slow changes in the total regulator concentration that appear when the degradation rates differ.

B. The independence measure

We will now try to gain some insight into why underlying independent ligand bindings do not necessarily lead to non-cooperative activation. The quasistatic approximations (fast on/off rates compared to turnovers) for Eqs. (4)-(6) lead to [16];

$$r_2 = \frac{K_3K_4}{K_3K_4 + 2K_4s + s^2}\; r_d \tag{24}$$

$$r_3 = \frac{2K_4s}{K_3K_4 + 2K_4s + s^2}\; r_d \tag{25}$$

$$r_4 = \frac{s^2}{K_3K_4 + 2K_4s + s^2}\; r_d \tag{26}$$

$$r_d = r_2 + r_3 + r_4 \tag{27}$$

being satisfied at all times.

If we define the geometric average of the two dissociation constants, $K_{34} = (K_3K_4)^{1/2}$, and the underlying independence measure, $\zeta_{34} = \sqrt{K_4/K_3}$, r_4 may conveniently be rewritten as;

$$r_4 = \frac{s^2}{K_{34}^2 + 2\zeta_{34}\,K_{34}\,s + s^2}\; r_d \tag{28}$$

Therefore, for independent (underlying) ligand bindings, where $K_3 = K_4$ and $\zeta_{34} = 1$, r_4 may be rewritten as a product of two independent reactions;

$$r_4 = \frac{s^2}{(K_{34} + s)^2}\; r_d \tag{29}$$

For fully cooperative underlying bindings, where $K_4 \ll K_3$ and $\zeta_{34} = 0$, we get;

$$r_4 = \frac{s^2}{K_{34}^2 + s^2}\; r_d \tag{30}$$

By analogy between Eqs. (1) and (28), this provides support for the interpretation that $\zeta_e = 1$ may be read as "independent" ligand bindings and $\zeta_e = 0$ may be read as "cooperative" as stated in Eq. (2).

However, if r_d depends on the ligand concentration, s, the independence in the underlying need not be inherited by the overall reaction. We will now look into, when this is the case. For simplicity, let us assume that all dimer forms of the regulator are equally protected against proteolytic degradation, i. e. $\lambda_2 = \lambda_3 = \lambda_4 = \lambda_d$. In the static limit, the total dimer concentration may be written;

$$r_d = \frac{b_1 - \lambda_1 r_1}{2\lambda_d} \tag{31}$$

If the dimers are stable (k_2^-=0), the numerator is a constant, and so is r_d. Thus, from Eq. (28), we conclude that the independent binding in the underlying reaction is inherited by the overall reaction. However, if the dimers are unstable (k_2^->0), the dimer concentration becomes a function of the ligand concentration, s. We shall see that, in order for Eq. (1) to represent the coupled processes, we can remedy this by allowing for a modified effective dissociation constant and the independence, ζ_e, to be below 1. Similarly, if the active dimer has lower proteolytic degradation than the other dimer forms, r_d will depend on the ligand concentration and, again, this leads to modified effective independence.

C. Cooperativity by binding to cognate operator (DNA) site

So far, we have investigated the production and activation of the transcription factor through ligand binding. Let us now consider what happens when the activated transcription factor binds to its cognate operator site on the DNA as indicated in Fig. 1. Here, we consider this process in quasistatic equilibrium (fast on/off rates) and therefore the concentration of occupied DNA sites is;

$$p_a = \frac{r_a}{K_x + r_a} p_t \tag{32}$$

where p_a/p_t is the fraction of operator sites occupied by TFs and $K_x = k_x^-/k_x^+$ is the dissociation constant for the process. Insertion of r_a from Eq. (1) and rearranging results in;

$$p_a = \frac{s^2}{s^2 + 2\tilde{\zeta}_e\tilde{K}_e s + \tilde{K}_e^2}\;\; \frac{r_m}{r_m + K_x}\; p_t \tag{33}$$

which is of the same functional form as the activated regulator concentration, but now with appropriately

rescaled independence measure and dissociation constant;

$$\tilde{\zeta}_e = \sqrt{\frac{K_x}{r_m + K_x}}\ \zeta_e \tag{34}$$

$$\tilde{K}_e = \sqrt{\frac{K_x}{r_m + K_x}}\ K_e \tag{35}$$

High protein levels, $r_m \gg K_x$, will push the activation towards cooperative behavior, i. e. $\tilde{\zeta}_e \ll \zeta_e$. This criterion is easily satisfied with typical experimentally observed dissociation constants and regulator concentrations [17].

This way of generating cooperative activation generalizes to higher order activation. Furthermore, the activation in downstream first order processes resulting from the activation will look increasingly cooperative for each step. In this sense, cooperative activation constitutes a stable fixed point as a functional form. We express this symbolically as;

$$\frac{s^n}{(K+s)^n} \longrightarrow \text{const} \times \frac{s^n}{\tilde{K}^n + s^n} \tag{36}$$

where the arrow signifies convergence for large activating regulator concentrations or higher number of first order steps in the sequence of processes. For example, even for 4th order activation, essentially full cooperativity is easily established with a single activation followed by a first order process.

In literature, the parametrization $r_a = s^h/(K_e^h + s^h)\ r_m$ is frequently used as a proxy for the TF-ligand concentration. However, it fails to simultaneously reproduce the asymptote at $s \ll K_e$ and the behavior around $s \sim K_e$. Further, it fails to capture the convergence towards cooperative binding as in Eqs. (34) and (36).

RESULTS AND DISCUSSION

We have presented two mechanisms that make the sensing of a ligand through activation of TFs and subsequent binding to their cognate promoter site on the DNA appear cooperative. The apparent cooperativity of the activation of the dimeric TFs was found to be possible in systems where dimerization drives ligand binding with unstable dimers. The apparent cooperativity generated when activated TFs bind to their cognate site on the DNA only requires the maximal TF concentration to be higher than the dissociation constant for the binding to DNA.

Both mechanisms can be seen as a consequence of having two saturation processes on top of each other. When the dimer is unstable, the initial balance between the monomer form and the dimer form will be shifted towards the dimer form as the ligand concentration increases. This saturation process happens in parallel with the underlying shifting between the R_2 form and ligand bound R_2S and R_2S_2 forms of the TF. As a result, the effective dissociation constant shifts to higher values than the underlying, $K_e > K_{34}$. Therefore, at the effective dissociation constant, the numerator in Eq. (26) has already shifted to s^2 behavior. This leads to the observed cooperative behavior. With this clarification, we expect the mechanism to generalize to higher order activation of TFs. The explanation for the cooperative behavior as the active TF binds on the DNA is similar, except, here the effective dissociation constant shifts to lower values, but again with the effect that the independence is quenched at ligand concentration approximating to the effective dissociation constant.

In Fig. 2, two examples with independent underlying ligand bindings are shown. As can be seen from the plots in the upper panels, both display cooperative behavior in the activation of the TF. Figs. 2a-b are log-linear plots, stressing the behavior around K_e. The log-log plots in Figs. 2c-d provide a representation of the full dynamic range of the activation and give a clear view of the asymptotic behavior. The parameters used in the calculations are shown in Table I (indicated as "cooperative") and are all within the referenced physiological ranges. In the lower panels, the time response for the two "cooperative" parameter sets are shown. This demonstrates, that the system is structured such that through appropriate evolution of parameters it can effect both slow and fast responses to a sudden increase in the ligand concentration.

In Table I, we also show two examples which display non-cooperative behavior in the activation of the TF. They follow the behavior of the plots with effective independence ($\zeta_e = 1$) in Fig. 2. The left column has unstable dimers, whereas the right column has stable dimers, thereby covering typical situations. Both parameter sets lead to a fast response.

We note that it is possible to construct parameter sets with stable dimers and cooperative TF activation, though, with less typical proteolytic degradation rates ($\lambda_2 \gg \lambda_3 = \lambda_4$).

The cooperativity and the filtering properties of the TF activation depend on the basal production of regulator. Substituting the promoter driving expression of the TF by a heterologous promoter with stronger or weaker transcription rate will therefore result in altered cooperativity and filtering properties. Similarly, increasing the maximal regulator concentration, e.g., by placing the regulator on a multi-copy plasmid, will modify the overall cooperativity, the effective dissociation constant, and the response time of the regulatory motif.

We have given a couple of typical examples of regulatory systems which display cooperative behavior without requiring cooperativity at the elementary level. We expect that simple mechanisms similar to those presented here may apply to other regulatory systems as well.

III. CONCLUSION

The primary finding from our analysis is that the cooperative activation of transcription factors can be established even with *independent* underlying ligand bindings. The binding of the activated TF complex to its cognate promoter site on the DNA pushes the regulation even further in the direction of cooperative behavior. Our analysis accounts for the inherent cooperativity in the biological activity of many dimeric transcription factors.

A number of advantages are associated with the cooperative activation of transcription factors, and most are related to improvements of basic sensory function. Cooperative activation can provide an expansion of the dynamic range of gene expression, and it can improve stability when occurring in feed-back systems that control rapid switching between different responses. It is therefore important that we have modelling approaches that can account for cooperativity in sensory systems where underlying cooperative mechanisms may not be present, thus, explaining cooperativity without the need of elaborate evolutionary constraints.

Appendix: Ligand binding drives dimerization

For completeness, we include the simplest version of a regulatory motif where ligand binding drives dimerization

$$\xrightarrow{b_1} \mathrm{R} \qquad\qquad \mathrm{R} \xrightarrow{\lambda_1} \tag{A.1}$$

$$\mathrm{R} + \mathrm{S} \overset{k_2^{\pm}}{\rightleftarrows} \mathrm{RS} \qquad\qquad \mathrm{RS} \xrightarrow{\lambda_2} \tag{A.2}$$

$$2(\mathrm{RS}) \overset{k_4^{\pm}}{\rightleftarrows} \mathrm{R_2S_2} \qquad\qquad \mathrm{R_2S_2} \xrightarrow{\lambda_4} \tag{A.3}$$

The corresponding kinetic equations are

$$\frac{dr_1}{dt} = b_1 + k_2^- r_2 - k_2^+ r_1 s - \lambda_1 r_1 \tag{A.4}$$

$$\frac{dr_2}{dt} = k_2^+ r_1 s + 2k_4^- r_4 - 2k_4^+ r_2^2 - k_2^- r_2 - \lambda_2 r_2 \tag{A.5}$$

$$\frac{dr_4}{dt} = k_4^+ r_2^2 - k_4^- r_4 - \lambda_4 r_4 \tag{A.6}$$

with combinatorial weights explicitly included. We employ the convention, that reactions are numbered by the number of constituents in the resulting product.

Again, in the static limit of the activation, the form Eq. (1) provides an excellent description. To determine K_e we need the asymptotic behavior at small s and at large s in the static limit of the kinetic equations. The derivation is analogous to the derivation of Eqs. (11)-(19). With dissociation constants defined as;

$$K_2 = \frac{k_2^- + \lambda_2}{k_2^+} \approx \frac{k_2^-}{k_2^+} \tag{A.7}$$

$$K_4 = \frac{k_4^- + \lambda_4}{k_4^+} \approx \frac{k_4^-}{k_4^+} \tag{A.8}$$

the asymptotic behavior at small s is given by;

$$r_4 = \frac{r_{1_{\max}}^2}{K_2^2 K_4} s^2 = \frac{b_1^2}{\lambda_1^2 K_2^2 K_4} s^2 \tag{A.9}$$

$$r_{1_{\max}} = \frac{b_1}{\lambda_1} \tag{A.10}$$

The maximal RS and activated regulator levels are reached at large s;

$$r_{2_{\max}} = \frac{\lambda_2}{4\lambda_4} K_4 \left(\sqrt{1 + \frac{8 b_1 \lambda_4}{K_4 \lambda_2^2}} - 1 \right) \tag{A.11}$$

$$r_m = r_{4_{\max}} = \frac{r_{2_{\max}}^2}{K4} \tag{A.12}$$

The effective dissociation constant sits at the crossing of the asymptotes;

$$K_e = \frac{K_2}{r_{1_{\max}}} \sqrt{K_4 r_{4_{\max}}} = K_2 \frac{r_{2_{\max}}}{r_{1_{\max}}} \tag{A.13}$$

and is typically different from K_2.

In the limit of low- or high-level production of regulator we find;

$$K_e^2 = \begin{cases} \frac{\lambda_1^2}{\lambda_2^2} K_2^2 & \frac{8 b_1 \lambda_4}{K_4 \lambda_2^2} \ll 1 \\ \frac{K_4 \lambda_1^2}{2\lambda_4 b_1} K_2^2 & \frac{8 b_1 \lambda_4}{K_4 \lambda_2^2} \gg 1 \end{cases} \tag{A.14}$$

In the case where the production of regulator is low and the monomer forms have similar degradation rates, this does not lead to a modification of the dissociation constant. However, when there is large production of regulator, the rescaling of the dissociation constant can be significant. In the case where ligand binding drives dimerization, we did not find cooperativity in the activation with physiological values of the parameters that we tested. However, the transcriptional activation at the promoter may still be cooperative as a consequence of the mechanism described in "Cooperativity by binding to cognate operator (DNA) site".

[1] C. Fuqua, S. C. Winans, and E. P. Greenberg, Annual Review of Microbiology **50**, 727 (1996).
[2] P. Williams, Expert Opin. Ther. Targets **6**, 257 (2000).
[3] A. Eberhard, J. Bacteriology **109**, 1101 (1972).
[4] K. H. Nealson, T. Platt, and J. W. Hastings, J. Bacteriol. **104**, 313 (1970).
[5] S. H. Choi and E. P. Greenberg, J. Bacteriology **174**, 4064 (1992).
[6] J. Zhu and S. C. Winans, Proc. Natl. Acad. Sci. USA **96**, 4832 (1999).
[7] J. Zhu and S. C. Winans, Proc. Natl. Acad. Sci. USA **98**, 1507 (2001).
[8] J. E. Gonzalez and N. D. Keshavan, Microbiology & Molecular Biology Reviews **70**, 1 (2006).
[9] M. J. Bottomley, E. Muraglia, R. Bazzo, and A. Carfi, Journal of Biological Chemistry **282**, 13592 (2007).
[10] K. J. Sappington, A. A. Dandekar, K.-I. Oinuma, and E. P. Greenberg, mBio **2**, e00011 (2011).
[11] A. Claussen, T. H. Jakobsen, T. Bjarnsholt, M. Givskov, M. Welch, J. Ferkinghoff-Borg, and T. Sams, International Journal of Molecular Sciences **14**, 13360 (2013).
[12] A. V. Hill, Proceedings of the Physiological Society , iv (1910).
[13] C. Bohr, Zentralblatt für Physiologie **XVIII**, 682 (1904).
[14] C. Bohr, K. Hasselbalch, and A. Krogh, Skandinavisches Archiv Für Physiologie **16**, 402 (1904).
[15] J. Ferkinghoff-Borg and T. Sams, Mol. BioSyst. **10**, 103 (2014).
[16] M. Welch, J. Gross, J. T. Hodgkinson, D. R. Spring, and T. Sams, Biochemistry **52**, 4433 (2013).
[17] K. Sneppen, *Models of Life* (Cambridge University Press, Cambridge, 2014).
[18] M. Fagerlind, S. Rice, P. Nilsson, M. Harlen, S. James, T. Charlton, and S. Kjelleberg, Journal of Molecular Microbiology and Biotechnology **6**, 88 (2003).
[19] M. G. Fagerlind, P. Nilsson, M. Harlén, S. Karlsson, S. A. Rice, and S. Kjelleberg, BioSystems **80**, 201 (2005).
[20] M. Schlosshauer and D. Baker, Protein Sci **13**, 1660 (2004), 0131660[PII], 15133165[pmid].
[21] M. Schlosshauer and D. Baker, J. Phys. Chem. B **106**, 12079 (2002).

TABLE I. Parameters used in the model where dimerization drives ligand binding in Fig. 2. The two leftmost parameter sets lead to cooperative activation at the promoter as shown in Fig. 2. The "Low Pass" set has slow dimer dilution and fast monomer dilution which gives rise to a noise-reducing slow response in TF activation, i.e. analogous to low-pass filters. The "All Pass" set has equal dimer and monomer dilution rates which lead to all-pass characteristics, i.e. a fast response. The dilution rates include proteolytic degradation as well as dilution by cell division. The two rightmost parameter sets lead to non-cooperative activation at the promoter, one with unstable dimer, the other with stable dimer.

	Cooperative		**Non-cooperative**			
Par	**Low Pass**	**All Pass**	**Unstable R_2**	**Stable R_2**	**Description**	**Ref.**
	Fig. 2, left	Fig. 2, right			Illustration	
b_1	1000 nM/h	1000 nM/h	1000 nM/h	1000 nM/h	Constitutive regulator production	[18, 19]
k_2^+	0.05 $\mathrm{nM^{-1}h^{-1}}$	0.05 $\mathrm{nM^{-1}h^{-1}}$	0.05 $\mathrm{nM^{-1}h^{-1}}$	7.2 $\mathrm{nM^{-1}h^{-1}}$	$2R \rightarrow R_2$ rate constant	[19–21]
k_2^-	100 $\mathrm{h^{-1}}$	4000 $\mathrm{h^{-1}}$	100 $\mathrm{h^{-1}}$	0 $\mathrm{h^{-1}}$	$2R \leftarrow R_2$ rate	[17, 19]
k_3^+	100 $\mathrm{nM^{-1}h^{-1}}$	100 $\mathrm{nM^{-1}h^{-1}}$	10 $\mathrm{nM^{-1}h^{-1}}$	10 $\mathrm{nM^{-1}h^{-1}}$	$R_2 + S \rightarrow R_2S$ rate constant	[19]
k_3^-	1000 $\mathrm{h^{-1}}$	1000 $\mathrm{h^{-1}}$	1000 $\mathrm{h^{-1}}$	1000 $\mathrm{h^{-1}}$	$R_2 + S \leftarrow R_2S$ rate	[19]
k_4^+	100 $\mathrm{nM^{-1}h^{-1}}$	100 $\mathrm{nM^{-1}h^{-1}}$	10 $\mathrm{nM^{-1}h^{-1}}$	10 $\mathrm{nM^{-1}h^{-1}}$	$R_2S + S \rightarrow R_2S_2$ rate constant	[19]
k_4^-	1000 $\mathrm{h^{-1}}$	1000 $\mathrm{h^{-1}}$	1000 $\mathrm{h^{-1}}$	1000 $\mathrm{h^{-1}}$	$R_2S + S \leftarrow R_2S_2$ rate	[19]
λ_1	20 $\mathrm{h^{-1}}$	10 $\mathrm{h^{-1}}$	0.5 $\mathrm{h^{-1}}$	20 $\mathrm{h^{-1}}$	Monomer dilution rate	[6, 7]
λ_d	0.5 $\mathrm{h^{-1}}$	10 $\mathrm{h^{-1}}$	0.5 $\mathrm{h^{-1}}$	0.5 $\mathrm{h^{-1}}$	Dimer dilution rate	[8, 10, 11]
r_m	172 nM	19.1 nM	951 nM	847 nM	Maximal dimer concentration	
K_{34}	10 nM	10 nM	100 nM	100 nM	Underlying dissociation constant	
ζ_{34}	1	1	1	1	Underlying independence	
K_e	120 nM	125 nM	138 nM	100 nM	Effective dissociation constant	
ζ_e	0.09	0.15	0.91	1.00	Effective independence	

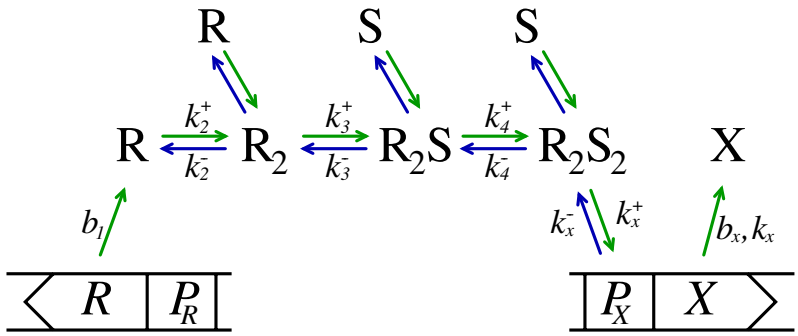

FIG. 1. **Activation of transcription factors**
Schematic diagram of the production, dimerization, and activation of a dimeric transcription factor (TF). The timeline for forward reactions reads from left to right. The binding of the activated transcription factor, R_2S_2, to a cognate operator site on the DNA for controlled transcription of a target gene, X, is shown too. On-rate constants are superscripted "+" whereas off-rates are superscripted "−". The basal synthetase of the monomer TF is denoted b_1. The overall role of the motif is to control the expression of the product X in response to changing concentrations of the ligand S. At low concentrations of active TFs, the transcription of X proceeds at background rate, b_x, and shifts to k_x at high concentrations of active TFs. In the manuscript, the concentrations (not shown) of constituents are r_1=[R], r_2=[R_2], r_3=[R_2S], and r_4=[R_2S_2]. The total concentration of promoter sites on the DNA is denoted p_t=[P_X] while the concentration of activated sites is p_a. All forms of the TFs may be subject to proteolytic degradation (not shown).

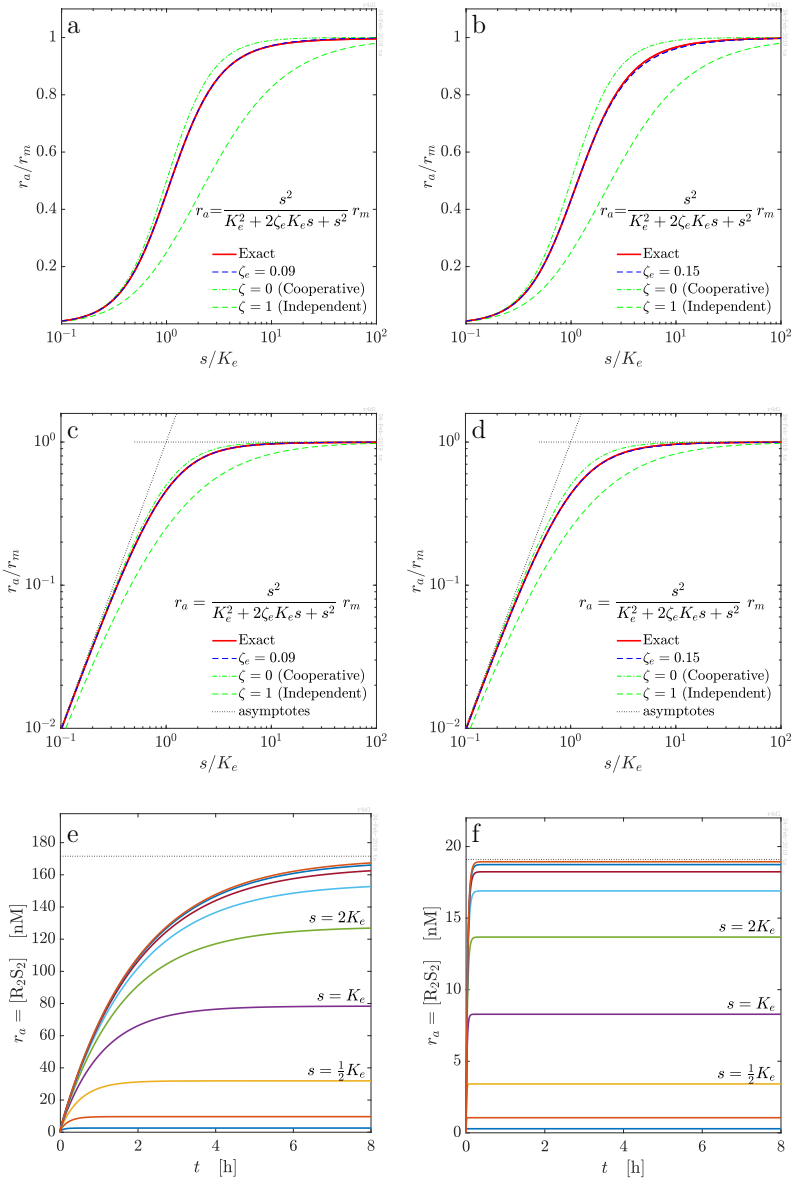

FIG. 2. **Cooperative activation of dimeric regulator**
Activated regulator concentration as a function of ligand concentration for the model where dimerization drives ligand binding with parameter sets from Table I. Semi-log (panels a and b) as well as log-log (panels c and d) versions are shown. Slow response is shown in panels a, c, e. Fast response is shown in panels b, d, f. Panels a, c and b, d: We observe that TF activation appears almost fully cooperative even though the underlying ligand bindings are independent. Panels c) and d): Time response following introduction of the ligand at concentrations $s = 2^n K_e$, $n = -3, -2, ..., 5$.