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

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Cooperative behaviour 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. The resulting phenotypic "switch" is often very abrupt, and has all the hallmarks of being underpinned by a highly cooperative mechanism. One ongoing controversy is 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 [1–6]. While looking into the differences between these contrasting mechanisms, we noted that driving of the system by continuous turnover of the TF leads to a rescaled dissociation constant and increased cooperativity. This is consistent with the experiment reported by Claussen and coworkers [6]. 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 [7–9]. 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;

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

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

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 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

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in bi-stable biological switches [10]. We shall primarily be concerned with the typical case where homodimerization drives ligand binding. However, a brief account of the case where ligand binding precedes dimerization is also 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;

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

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

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

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

as illustrated in Fig. 1. Here, we denote the constitutive production of regulator as b_1. 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) we need the asymptotic behaviour at small and large values of s for the second order process. On a logarithmic scale, these are straight lines (of slope 2 and 0 respectively) and the effective dissociation constant sits at their crossing. 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 behaviour. 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;

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

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

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

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 parameterisation, 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) for Eqs. (5)-(6) lead to;

$$r_4 = \frac{s^2}{K_3K_4 + 2K_4 s + s^2}\, r_d \tag{24}$$

$$r_d = r_2 + r_3 + r_4 \tag{25}$$

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, $\zeta_{34} = \sqrt{K_4/K_3}$, this may conveniently be rewritten as;

$$r_4 = \frac{s^2}{K_{34}^2 + 2\zeta_{34}\, K_{34}\, s + s^2}\, r_d \tag{26}$$

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;

$$r_4 = \frac{s^2}{(K_{34} + s)^2}\, r_d \tag{27}$$

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

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

By analogy between Eqs. (1) and (26), 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;

$$r_d = \frac{b_1 - \lambda_1 r_1}{2\lambda_d} \tag{29}$$

If the dimers are stable (k_2^-=0), the numerator is a constant, and so is r_d. Thus, 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;

$$p_a = \frac{r_a}{K_x + r_a} p_t \tag{30}$$

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;

$$p_a = \frac{s^2}{s^2 + 2\tilde{\zeta}_e \tilde{K}_e s + \tilde{K}_e^2} \quad \frac{r_m}{r_m + K_x}\, p_t \tag{31}$$

which is of the same functional form as the activated regulator concentration, but now with appropriately rescaled independence measure and dissociation constant;

$$\tilde{\zeta}_e = \sqrt{\frac{K_x}{r_m + K_x}}\, \zeta_e \tag{32}$$

$$\tilde{K}_e = \sqrt{\frac{K_x}{r_m + K_x}}\, K_e \tag{33}$$

High protein levels, $r_m \gg K_x$, will push the activation towards cooperative behaviour, i. e. $\tilde{\zeta}_e \ll \zeta_e$. This criterion is easily satisfied with typical experimentally observed dissociation constants and regulator concentrations [11].

This way of generating cooperative activation generalises 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;

$$\frac{s^n}{(K + s)^n} \longrightarrow \text{const} \times \frac{s^n}{\tilde{K}^n + s^n} \tag{34}$$

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 the literature, the parameterisation $r_a = s^h/(K_e^h + s^h)\; r_m$ 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. (32) and (34).

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.

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 behaviour in the activation of the TF. Figs. 2a-b are log-linear plots, stressing the behaviour 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 behaviour. 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 behaviour in the activation of the TF. They follow the behaviour 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 ($\lambda_2 \gg \lambda_3 = \lambda_4$).

III. CONCLUSION

The primary finding from our analysis is that cooperative activation of transcription factors can quite easily be established even with *independent* underlying ligand bindings. The binding of the activated TF to its cognate promoter site on the DNA pushes the regulation even further in the direction of cooperative behaviour.

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 such as that from the lac operon may therefore result in altered cooperativity and filtering properties. Similarly, increasing the maximal regulator concentration, e.g., by placing the regulator on a multicopy plasmid, may modify the overall cooperativity, the effective dissociation constant, and the response time of the motif.

Appendix: 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 behaviour 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 behaviour 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;

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

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] J. Zhu and S. C. Winans, Proc. Natl. Acad. Sci. USA **96**, 4832 (1999).

[2] J. Zhu and S. C. Winans, Proc. Natl. Acad. Sci. USA **98**, 1507 (2001).

[3] J. E. Gonzalez and N. D. Keshavan, Microbiology & Molecular Biology Reviews **70**, 1 (2006).

[4] M. J. Bottomley, E. Muraglia, R. Bazzo, and A. Carfi, Journal of Biological Chemistry **282**, 13592 (2007).

[5] K. J. Sappington, A. A. Dandekar, K.-I. Oinuma, and E. P. Greenberg, mBio **2**, e00011 (2011).

[6] 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).

[7] A. V. Hill, Proceedings of the Physiological Society , iv (1910).

[8] C. Bohr, Zentralblatt für Physiologie **XVIII**, 682 (1904).

[9] C. Bohr, K. Hasselbalch, and A. Krogh, Skandinavisches Archiv Für Physiologie **16**, 402 (1904).

[10] J. Ferkinghoff-Borg and T. Sams, Mol. BioSyst. **10**, 103 (2014).

[11] K. Sneppen, *Models of Life* (Cambridge University Press, Cambridge, 2014).

[12] M. Fagerlind, S. Rice, P. Nilsson, M. Harlen, S. James, T. Charlton, and S. Kjelleberg, Journal of Molecular Microbiology and Biotechnology **6**, 88 (2003).

[13] M. G. Fagerlind, P. Nilsson, M. Harlén, S. Karlsson, S. A. Rice, and S. Kjelleberg, BioSystems **80**, 201 (2005).

[14] M. Schlosshauer and D. Baker, Protein Sci **13**, 1660 (2004), 0131660[PII], 15133165[pmid].

[15] 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	Regulator production rate	[12, 13]
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	[13–15]
k_2^-	100 $\mathrm{h^{-1}}$	4000 $\mathrm{h^{-1}}$	100 $\mathrm{h^{-1}}$	0 $\mathrm{h^{-1}}$	$2R \leftarrow R_2$ rate	[11, 13]
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	[13]
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	[13]
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	[13]
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	[13]
λ_1	20 $\mathrm{h^{-1}}$	10 $\mathrm{h^{-1}}$	0.5 $\mathrm{h^{-1}}$	20 $\mathrm{h^{-1}}$	Monomer dilution rate	[1, 2]
λ_d	0.5 $\mathrm{h^{-1}}$	10 $\mathrm{h^{-1}}$	0.5 $\mathrm{h^{-1}}$	0.5 $\mathrm{h^{-1}}$	Dimer dilution rate	[3, 5, 6]
K_d	10 nM	10 nM	100 nM	100 nM	Underlying dissociation constant	
ζ_d	1	1	1	1	Independence	
r_m	172 nM	19.1 nM	951 nM	847 nM	Maximal dimer concentration	
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	

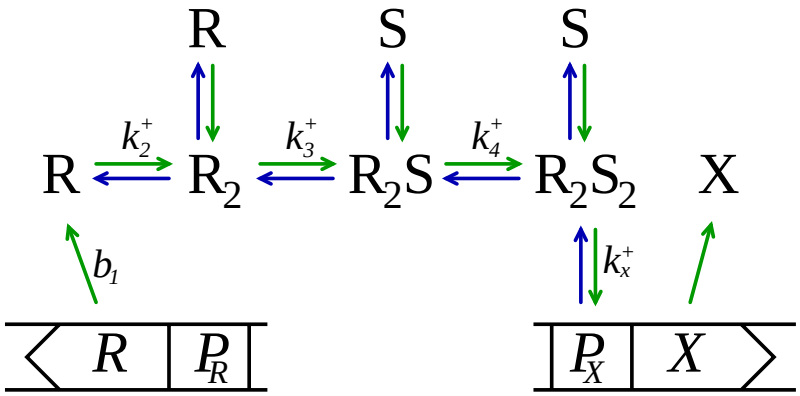

FIG. 1. **Activation of transcription factors**
Schematic diagram of the production, dimerization and activation of a (dimeric) transcription factor (TF). The binding of the activated transcription factor to a cognate operator site on the DNA for controlled transcription of a target gene, X, is shown too. The different forms of the TFs are subject to proteolytic degradation (not shown).

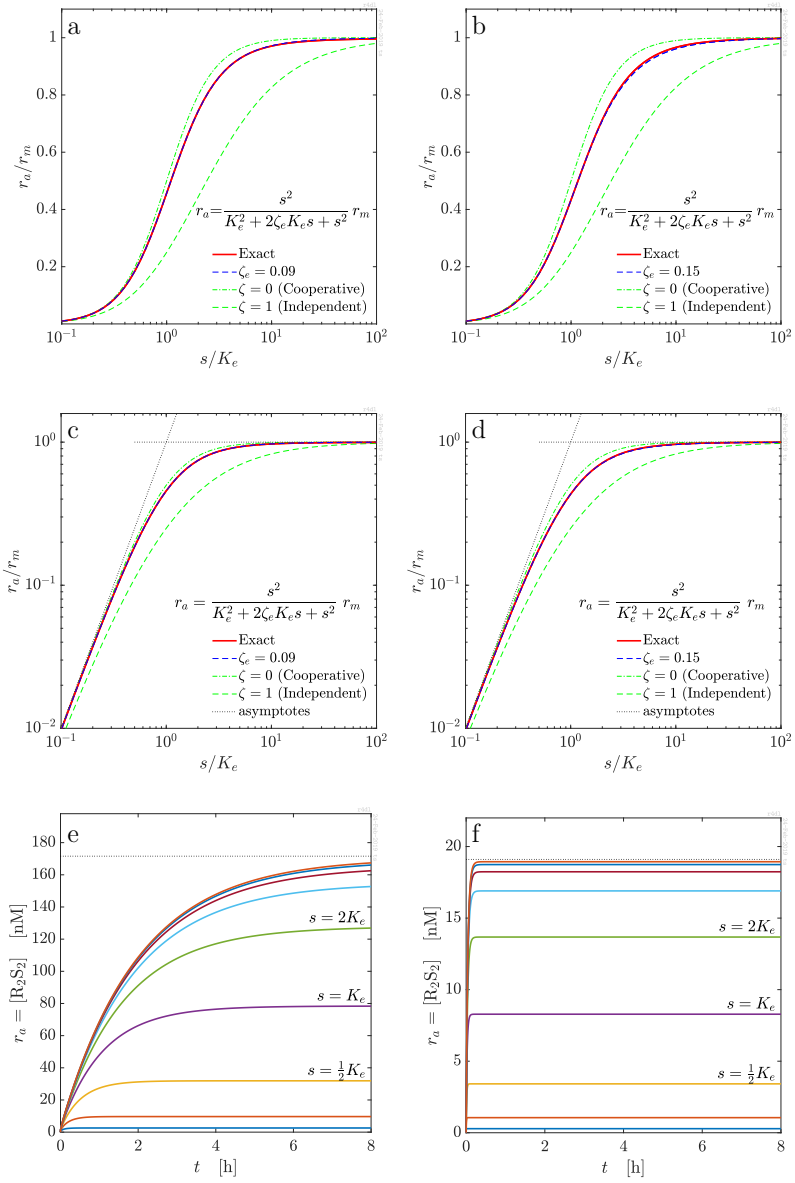

FIG. 2. **Cooperative activation of dimeric regulator**
Activated regulator concentration as a function of ligand concentration for the model where dimerisation drives ligand binding with parameter sets from Table I. Semi-log (panels a and b) as well as log-log (panels c and d) versions are shown. Slow 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 $s = 2^n K_e$, $n = -3, -2, ..., 5$.