

Supplementary Materials: Thermodynamic Modelling of Transcriptional Control: A Sensitivity Analysis

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This file collects all the model derivations and mathematical proofs justifying the results stated in the work “Thermodynamic Modelling of Transcriptional Control: A Sensitive Analysis”.

Bibliographic references in this document are independent from those of the main manuscript.

S1. Model Derivation

In this section, we will deduce brand-new expressions of the BEWARE operator. We will work in a general framework, where we will assume that the gen expression is controlled by a general number of cooperative transcription factors (TFs). In the literature, both competition and cooperativity have been determinant factors that tend to convolute greatly the mathematical expressions, even in the most simple case of two transcription factors. Hence, in order to get to the right expression of the BEWARE operator, we first need to declare briefly which are the model assumptions and, from there, how the competition and cooperativity are defined. Let us consider a promoter of a gene p controlled transcriptionally by the TFs $\mathcal{T} = \{T_1, \dots, T_M\}$ by binding competitively to a module of n enhancers. We consider that some of the TFs will increase the production rates of the protein P while the rest try to repress the same rates. Thus, \mathcal{T} is divided into two different families, M_a activators $\mathcal{T}_A = \{T_1, \dots, T_{M_a}\}$ and $M_r = M - M_a$ repressors $\mathcal{T}_R = \{T_{M_a+1}, \dots, T_M\}$ such that $\mathcal{T} = \{\mathcal{T}_A, \mathcal{T}_R\}$. The goal of the statistical thermodynamic model is to describe the synthesis rate of P in terms of the TFs concentrations and their activator/repressor role, that is,

$$\frac{d[P]}{dt} = \text{BEWARE}([T_1], \dots, [T_M]) \quad (\text{S1})$$

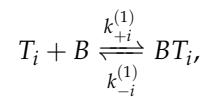
where “BEWARE()” is the function specifying the dependence on the TFs, and is subjected to the biochemical mechanisms involved in all these processes. Our aim in this section is to show a methodology for deriving explicit simple analytical expressions for the BEWARE operator by using thermodynamic modelling. Although it is out of our main goals, let us remark that the right-hand side of model (S1) is usually accompanied with a degradation contribution, $-\beta[P]$, β being a degradation rate [1].

As mentioned in the introductory section, in this work, we will distinguish between models based on either recruitment or stimulated mechanisms assumed for the RNA Polymerase (RNAP). In order to clarify the difference between these two models, we enumerate in the next paragraphs the main assumptions that are used in order to develop them. We also outline in Figure 1 in the main manuscript the biochemical mechanisms, which are mainly related with affinity, cooperativity or the manner in which TFs control transcriptional activity.

S1.1. Thermodynamic Description of Microscopic Configurations: Assumptions

H1) *Separated time scales*: The reactions driving transcriptional control are much more faster than the changes in TF concentrations and the synthesis of the protein P . Thus, TFs/RNAP binding in enhancers/promoter will be considered in thermodynamical equilibrium given by the Law of Mass Action [2]. For instance, in chick embryo neural tube Shh signalling, it has been pointed out that changes in Gli protein concentrations take place at a timescale of days compared to mRNA variations in timescales of minutes or hours [1,3].

The binding of a TF T_i to one of the n free binding sites, B , can be interpreted as the chemical reaction



where the Law of Mass Action establishes that the complex BT_i has a concentration at equilibrium given by

$$[BT_i] = \frac{k_{+i}^{(1)}}{k_{-i}^{(1)}} [T_i][B] := \frac{[T_i]}{K_i^{(1)}} [B].$$

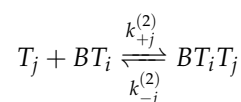
In the notation proposed, $K_i^{(1)} = k_{-i}^{(1)} / k_{+i}^{(1)}$ is the dissociation constant of the reaction, with units of concentration so that the quotient $\frac{[T_i]}{K_i^{(1)}}$ is dimensionless. The superscript (1) stands for the dissociation constant of a reaction that takes place in the absence of another TF previously bound in the enhancer module. The dissociation constants quantify the affinity of the TFs for their binding sites, being more affine those TFs with lower dissociation constants. The binding of a RNA polymerase in a free promoter follows the same rule, whose concentration at equilibrium is

$$[BRNAP] = \frac{k_{+RP}}{k_{-RP}} [RNAP][B] := \frac{[RNAP]}{K_{RP}} [B].$$

Please note that K_{RP} does not need any superscript since we are working with biological modules that are controlled by n enhancers but only one promoter, hence the maximum number of bound RNA polymerases is reduced to one. In Figure 1, in the main manuscript, these admissible bounds are indicated by black doubled sided arrows.

- H2) *TFs binding sites, B , are constituted by n identical sites that can be occupied competitively by any TF.* The basic rule of this competition is that the dissociation constant of the free sites configuration does not depend on their position but might depend on other existing bound TFs in the same module.

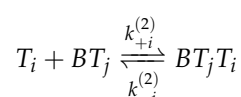
Let us suppose the reaction of the binding between a molecule T_i and a free enhancer configuration B occurred. Then, if a second molecule T_j binds to this configuration, we have



with respective equilibrium concentration

$$[BT_i T_j] = \frac{[T_i][T_j]}{K_i^{(1)} K_j^{(2)}} [B]$$

where now the superscript (2) denotes the dissociation constant for a reaction of a TF that binds the operator with already one TF in some other site. Let us note that previous expression is independent of the enhancers occupied since we are considering them identical. Note that the product could be also obtained by simply changing the order of linkage of the TFs, i.e.,



although the corresponding concentration could not be the same as we explain in the next paragraph.

In biological literature [4–7], it has been observed that, in this competition for the enhancers, a well known mechanism could be involved, called cooperativity, that assumes that one ligand supports the binding of others [8]. Thus, we will call non cooperative to TFs all those proteins whose enhancer's affinities are not modified because of any previously bound TFs, that is, they verify $K_l^{(2)} = K_l^{(1)}$ for all $l = 1, \dots, M$. If that is the case, it is plausible to assume the same relation for later bindings, that is, $K_l^{(m)} = K_l^{(1)}$ for $m \geq 2$, and, as a consequence of this sequential independence, we will denote the dissociation constant by K_l skipping the superscript. Then, if all the TFs under consideration are non-cooperative, we easily deduce that the concentration at equilibrium of a generic configuration with j_i proteins of the T_i species is

$$[BT_1^{j_1} \dots T_M^{j_M}] = [B] \prod_{i=1}^M \left(\frac{[T_i]}{K_i} \right)^{j_i} \quad (S2)$$

independently of the sequential order of binding and of the specific positions occupied for the TFs. Let us recall that, since n denotes the total number of free binding sites for TFs, then $\sum_{i=1}^M j_i \leq n$ has to be verified. $j_0 = n - \sum_{i=1}^M j_i (\geq 0)$, subsequently, will denote the number of free spaces in the configuration.

Cooperativity, or anti-cooperativity, occurs when the existence of another previously bound protein, T_i , affects the affinity of the new binding protein T_j (cooperativity is represented graphically by dotted arrows in Figure 1 in the main text. If the binding process of a protein T_j is facilitated by another already bound T_i protein, TF-TF cooperativity, this can be modelled by considering:

$$K_j^{(2)} = K_j^{(1)} / c \quad \text{being } c > 1.$$

Anti-cooperativity can also occur when the bound protein impedes the binding of the protein T_j , which can also be modelled by the previous expression where c is now a positive constant smaller than 1. Since the only difference between cooperativity and anti-cooperativity is a threshold value for c , in the subsequent considerations about modelling, we will refer to the constant c and not distinguish between both cases.

If cooperativity occurs in the presence of multiple TFs, it would be necessary to know which TFs are affected by other TFs, since the equilibrium concentration will depend on these relations. Regarding this question in the literature, several options have been considered. Partial cooperativity [5] would occur when the existence of a specific TF, T_i , modifies equally the affinity of any posterior transcription factor binding of the same family, which is $K_i^{(m)} = K_i^{(1)} / c_i$ for $m \geq 2$. Total cooperativity [7] would occur when the presence of any bound TF, T_i , modifies the affinity of any posterior binding in the same manner, i.e., $K_j^{(m)} = K_j^{(1)} / c$ for $m \geq 2$ and $j = 1, \dots, M$. These relations have been represented in Figure 1 in the main text with red dotted arrows. Then, by direct adaptation of the previous considerations, we have that

$$[BT_1^{j_1} \dots T_M^{j_M}] = [B] c^{\left(\sum_{i=1}^M j_i - 1\right)_+} \prod_{i=1}^M \left(\frac{[T_i]}{K_i} \right)^{j_i} \quad (S3)$$

in the presence of total cooperativity while

$$[BT_1^{j_1} \dots T_M^{j_M}] = [B] \prod_{i=1}^M c_i^{(j_i - 1)_+} \left(\frac{[T_i]}{K_i} \right)^{j_i} \quad (S4)$$

if partial cooperativity for TFs occurs. Here, $(\cdot)_+$ denotes the positive part function needed because cooperativity is not present unless two or more cooperative TFs are

present in the configuration. Subsequently, we will denote by $\{\{T_1, \dots, T_M\}_c\}$ and $\{\{T_1\}_{c_1}, \dots, \{T_M\}_{c_M}\}$ the total and partial cooperativity, respectively. Let us observe that this notation covers the case of non cooperativity since it would correspond to the case $\{\{T_1, \dots, T_M\}_1\}$ or equivalently $\{\{T_1\}_1, \dots, \{T_M\}_1\}$. Since cooperativity has been described to cause deep changes in transcriptional logic [9], in this work, we present our results generalising both partial and total cases. The straightforward extension of total-partial cooperativity concepts is to consider that total cooperativity can occur only between some of the TFs, that is, between the elements of certain subsets of transcription factors. We will refer to this as mixed cooperativity, $\{\{T_1\}_{c_1}, \dots, \{T_N\}_{c_N}\}$, where \mathcal{T}_i denotes each of the N disjoint subgroups of TFs cooperating with cooperativity constant c_i . By analogue arguments, we obtain that, for mixed cooperativity,

$$[BT_1^{j_1} \dots T_M^{j_M}] = [B] \left(\prod_{i=1}^N c_i^{\left(\sum_{h \in \mathcal{T}_i} j_h - 1 \right)_+} \right) \left[\prod_{i=1}^M \left(\frac{[T_i]}{K_i} \right)^{j_i} \right], \quad (\text{S5})$$

where $\mathcal{I}_i = \{h; T_h \in \mathcal{T}_i\}$ is the set of subindexes of the TFs belonging to \mathcal{T}_i . Let us note that this approach of mixed cooperativity is new and, as far as the authors know, it has not been proposed in previous literature. As we will see in the next paragraphs, this generalization will become very handy since we can deduce the expressions of different cooperative cases from this description.

- H3) *The action of a bound TF is independent of the specific enhancer it is occupying, so the transcriptional contribution of configurations with the same number of TFs bound at different specific positions is the same.* Since the TFs compete for free enhancers, and there is not a predetermined binding order [10], multiple spatial configurations of occupied operators are allowed. Thus, in general, there is not a unique spatial distribution for a given configuration with a distribution of (j_1, \dots, j_M) bound transcription factors and j_0 free sites. For instance, if we consider $n = 3$, $M = 3$ and $j_1 = j_2 = j_3 = 1$, there are six possible spatial distributions with the same elements ($T_1 T_2 T_3$, $T_2 T_1 T_3$, $T_1 T_3 T_2$, $T_2 T_3 T_1$, $T_3 T_2 T_1$, $T_3 T_1 T_2$). In our description, spatial localisation of bound particles is not relevant, so, for a concrete configuration (j_1, \dots, j_M) and j_0 free sites, we will identify the $\frac{n!}{j_0! \prod_{i=1}^M j_i!}$ spatial different plausible configurations.

Assumption H2) and H3) describe the possible configurations of TFs bound to the binding sites. Let us observe that these assumptions not only imply the spatial but also the sequential independence of the equilibrium concentrations. As mentioned in the introductory section, our deduction separates now into two modelling versions: the recruitment and the stimulated approaches [11,12]. Our next hypotheses describe the RNA polymerases/promoter binding process in both versions.

- HR4) *Recruitment assumption: TFs work by bringing the transcriptional machinery by TFs/RNAP (anti-)cooperativity [12,13].* In [6], the synergy between a TF and RNAP is interpreted in terms of a “glue-like” interaction that would give rise to a modification of the RNA polymerase binding affinity modelled analogously to a TFs/RNAP cooperativity: each bound activator tries to pull the RNA polymerase in the promoter, modifying its affinity constant with a factor $\prod_{i=1}^{M_a} a_i^{j_i}$, where we denote $a_i > 1$ to the i -th *activator transcription intensity* for $i = 1, \dots, M_a$. On the other hand, in a symmetric manner, we can model the effect of $M - M_a$ repressors in terms of a “repulsive-like” interaction by modifying the RNAP binding affinity with a factor $\prod_{i=M_a+1}^M r_i^{j_i}$, where $r_i < 1$ is the i -th *repressor transcription intensity* for $i = M_a + 1, \dots, M$ in this case. Then, in general, the RNAP binding affinity will take the form

$$\frac{K_{RP}}{\prod_{i=1}^M t_i^{j_i}},$$

with $t_i = a_i > 1 \forall i = 1, \dots, M_a$ and $t_i = r_i < 1 \forall i = M_a + 1, \dots, M$. These TFs/RNAP cooperative type interactions are indicated by a blue dotted arrow in Figure 1 in the main text. Please note that this description is in concordance with the affinity definition: If the denominator is larger than one, then the RNA polymerase will be more affine. Since the denominator depends on the number of transcription factors and their activation/repression intensities, we are promoting or impeding the RNA polymerase cleavage, hence promoting or impeding the transcription itself.

HS4) *Stimulated assumption: Unaltered affinity of the RNA polymerase at the promoter*, that is, the binding affinity of the RNA polymerase to the promoter, K_{RP} , is invariant with respect to the bound TFs.

It is important to remark that only very few of the regulatory motifs shown in [6] match with the generality presented in this work. More concretely, the cases of simple repressor and activator coincide with the case of a single binding site, $n = 1$, and glue-like interaction for activators and total repression, $r = 0$, for repressors. This comes from the specific character of the binding sites considered in [6], i.e., one binding site can be only occupied by a unique kind of molecule, which does not allow the competition we are describing in this work.

Let us consider the multi-index $\vec{j} = (j_1, \dots, j_M) \in \mathbb{N}_0^M$ with j_i the number of bound TFs of the i -th species in the set of enhancers, j_0 the number of free sites and $j_P = 1$ if there is a bound RNA polymerase and $j_P = 0$, otherwise. In summary, we have that all the possible ways of obtaining an equilibrium concentration with (\vec{j}, j_P) TFs-RNAP bound is given by the *microstates*

$$Z^{(n)}(\vec{j}, j_P = 1; \mathcal{C}) = C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} [B] \left(\frac{[RNAP]}{K_{RP}} \right) \prod_{i=1}^M \left(\frac{t_i [T_i]}{K_i} \right)^{j_i}, \quad (S6)$$

$$Z^{(n)}(\vec{j}, j_P = 0; \mathcal{C}) = C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} [B] \prod_{i=1}^M \left(\frac{[T_i]}{K_i} \right)^{j_i},$$

if the recruitment approach, HR4), is assumed and

$$Z^{(n)}(\vec{j}, j_P; \mathcal{C}) = C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} [B] \left(\frac{[RNAP]}{K_{RP}} \right)^{j_P} \prod_{i=1}^M \left(\frac{[T_i]}{K_i} \right)^{j_i} \quad (S7)$$

when stimulated assumption HS4) is considered. In both cases, the variable \mathcal{C} describes the relation of cooperativity, if it exists, between the TFs. More concretely, by using (S3), (S4) and (S5), and the cooperativity function C takes the value

$$C(\mathcal{C} = \{T_1, \dots, T_M\}_c) = c^{\left(\sum_{i=1}^M j_i - 1 \right)_+}, \quad (S8)$$

when total cooperativity holds,

$$C(\mathcal{C} = \{\{T_1\}_{c_1}, \dots, \{T_M\}_{c_M}\}) = \prod_{i=1}^M c_i^{(j_i - 1)_+}, \quad (S9)$$

if partial cooperativity is verified, and finally

$$C(\mathcal{C} = \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}) = \prod_{i=1}^N c_i \left(\sum_{h \in \mathcal{I}_i} j_h^{-1} \right)_+ \quad (\text{S10})$$

when mixed cooperativity occurs. Let us observe that definitions (S6)-(S7) are absolutely consistent with the usual convention $0^j = 0$ when $j > 0$ because, in the absence of certain binding particles, it is impossible to obtain any configuration with those kinds of particles, and $0^0 = b^0 = 1$ with $b \geq 0$ since configurations with no bound particles of a certain type are independent of that substance concentration.

S1.2. Configuration Probability

Now, the thermodynamic methodology proposes to describe from previous calculations a probability for any possible configuration [14,15]. Let us notice that the sample space is determined by the multi-indices set

$$\Omega = \{(\vec{j}, j_P); \vec{j} \in \mathbb{N}_0^M, |\vec{j}| \leq n, j_P = 0, 1\},$$

where the constraint on $|\vec{j}| = \sum_{i=1}^M j_i \leq n$ is due to the limit of capacity of the n enhancers for accepting bound TFs. Now, using the description of all the possible configurations in terms of the concentrations of TFs and RNA polymerase, we define the probability of finding the module in a particular configuration of j_P RNA polymerase and j_1, \dots, j_M TFs with cooperativity function \mathcal{C} as

$$P^{(n)}(\vec{j}, j_P; \mathcal{C}) = \frac{Z^{(n)}(\vec{j}, j_P; \mathcal{C})}{\sum_{\{\vec{j}', j'_P\} \in \Omega} Z^{(n)}(\vec{j}', j'_P; \mathcal{C})}, \quad (\text{S11})$$

for all $(\vec{j}, j_P) \in \Omega$.

In the next subsections, these probabilities, corresponding to each microstate, will be averaged (BEWARE operator) according to the recruitment/stimulated transcription approaches.

S1.3. Recruitment BEWARE Operator

In the work of Shea et al [15], the BEWARE operator for the synthesis of a certain protein depends on the total probability of finding RNA polymerase in the promoter (i.e., proportional to the marginal distribution in the number of TFs evaluated at $j_P = 1$). This constitutes a new assumption, denoted by HR5, that has been widely used assuming recruitment assumption HR4 (see, for instance, [5,6]). We will denote by the recruitment BEWARE operator the function

$$\text{BEWARE}_r(\vec{T}, [\text{RNAP}]; \mathcal{C}) = C_B \sum_{|\vec{j}| \leq n} P^{(n)}(\vec{j}, j_P = 1; \mathcal{C})$$

where, in definition (S11), expression (S6) is assumed, and C_B is a proportionality constant. Let us note that C_B could depend on the control exerted in other enhancer modules or later stages of the whole genetic regulation process. Here, \vec{T} denotes a vector collecting the concentrations of all the TFs, that is, $\vec{T} = ([T_1], \dots, [T_M])$, and \mathcal{C} can be any of the cooperativity relations established in assumption (H2) whose contribution is determined by (S8)-(S10). Splitting the denominator in two sums, depending on the existence of RNA polymerase bound to the configuration, this expression can be easily rewritten in terms of the regulation factor function F_{reg} :

$$\text{BEWARE}_r(\vec{T}, [\text{RNAP}]; \mathcal{C}) = \frac{C_B}{1 + \frac{\sum_{|\vec{j}| \leq n} Z^{(n)}(\vec{j}, 0; \mathcal{C})}{\sum_{|\vec{j}| \leq n} Z^{(n)}(\vec{j}, 1; \mathcal{C})}} = \frac{C_B}{1 + \frac{K_{RP}}{[\text{RNAP}]_{F_{\text{reg}}(\vec{T}, \mathcal{C})}}} \quad (\text{S12})$$

where

$$F_{\text{reg}}(\vec{T}; \mathcal{C}) = \frac{\sum_{|\vec{j}| \leq n} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} \prod_{i=1}^M \left(\frac{t_i [T_i]}{K_i} \right)^{j_i}}{\sum_{|\vec{j}| \leq n} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} \prod_{i=1}^M \left(\frac{[T_i]}{K_i} \right)^{j_i}}. \quad (\text{S13})$$

Let us mention that activators and repressors play symmetric roles, mathematically speaking, since their activating/repressing nature is only reflected in the value of the parameter t_i , being bigger than 1 for activators and smaller than 1 for repressors.

S1.4. Stimulated BEWARE Operator

On the other hand, the stimulated transcription approach associates to any configuration with a bound RNA polymerase a transcription density [3,7,16]. We will call this new assumption HS5. In this point, we are going to follow the proposal of [7], adapted in later works [17,18], where activators and repressors have to be distinguished. Let us consider that $\vec{T}_A = ([T_1], \dots, [T_{M_a}]) \in \mathbb{R}^{M_a}$, $\vec{T}_R = ([T_{M_a+1}], \dots, [T_M]) \in \mathbb{R}^{M_r}$ the activators and repressors vectors, and $\vec{j}_A = (j_1, \dots, j_{M_a}) \in \mathbb{Z}_+^{M_a}$, $\vec{j}_R = (j_{M_a+1}, \dots, j_M) \in \mathbb{Z}_+^{M_r}$ the number of bound activators/repressors of the i -th specie, such that $M = M_a + M_r$. Considering the standard concatenation operator, we have that $\vec{T} = (\vec{T}_A, \vec{T}_R) \in \mathbb{R}^M$ and $\vec{j} = (\vec{j}_A, \vec{j}_R) \in \mathbb{Z}_+^M$.

Furthermore, it is necessary to fix the basal and maximal/minimal transcription levels definition:

- i) r_{bas} is a basal transcription rate one would expect from a completely empty configuration with no stimulated transcription at all. If basal transcription is not assumed, then $r_{bas} = 0$.
- ii) $(v_{max}^{(n)} + r_{bas})$: is the level of maximal transcriptional rate of the system, given by a configuration filled with n of the most powerful activator. The dependence of $v_{max}^{(n)}$ with respect to the total number of enhancers can be justified from the experiments developed in [5] (see Figure 4), where it was observed that a diminishing in the number of enhancers produces a reduction in the maximal expression levels.
- iii) $r_{bas} \tilde{r}_M^n$, with $\tilde{r}_M < 1$, the level of minimal transcriptional rate, that would correspond to the configuration completely bound to the most powerful repressors, assumed to be T_M .

From these basic levels and the probabilities $P^{(n)}$, given by (S11) and (S7), the activation or repression levels of all the possible configurations are determined by the following expressions:

- (i) for states with RNAP but no bound activators, that is $j_i = 0$ for any $i = 1, \dots, M_a$,

$$r_{bas} \left(\prod_{i=M_a+1}^M \tilde{r}_i^{j_i} \right) P^{(n)}(\vec{j}, j_P = 1; \mathcal{C}),$$

where $\tilde{r}_M \leq \tilde{r}_i < 1$ is a constant that stands for the repression strength of the i -th repressor.

- (ii) and, in the opposite case, that is states with some bound activators ($|\vec{j}_A| > 0$ that is $j_i > 0$ for some $i = 1, \dots, M_a$)

$$\left(\prod_{i=1}^{M_a} \tilde{a}_i^{j_i} \right) \left(\prod_{i=M_a+1}^M \tilde{r}_i^{j_i} \right) \left(r_{bas} + v_{max}^{(n)} \tilde{e}^{j_0 + \sum_{i=M_a+1}^M j_i} \right) P^{(n)}(\vec{j}, j_P = 1; \mathcal{C}),$$

where $\tilde{e} < \min\{\tilde{a}_i; i = 1, \dots, M_a\}$ is a constant of transcriptional efficiency proportional to free or repressor occupied enhancers, and $\tilde{a}_i \leq 1$ is a constant that stands for the activation strength of the i -th activator, $\tilde{a}_i = 1$ being in the case of the most powerful activator.

- (iii) If we assume that the level of transcription of configurations with the same number of activators and repressors should coincide independently of the total number of sites in the configuration (n), this allows us to conclude a plausible expression for

$$v_{max}^{(n)} = \frac{v_{max}^{(1)}}{\tilde{e}^{n-1}} \quad (\text{S14})$$

where $v_{max}^{(1)}$ is defined in terms of the maximal transcription when only one enhancer is available.

The synthesis of the protein under consideration depends, therefore, on the addition of all these transcriptional efficiencies of states with a bound RNAP, which, written in terms of the common factors r_{bas} and $v_{max}^{(n)}$, can be expressed as

$$\begin{aligned} \text{BEWARE}_s(\vec{T}, [\text{RNAP}]; \mathcal{C}) \\ = \frac{r_{bas}}{1 + \frac{K_{RP}}{[\text{RNAP}]}} \text{Basal}(\vec{T}; \mathcal{C}) + \frac{v_{max}^{(n)}}{1 + \frac{K_{RP}}{[\text{RNAP}]}} \text{Promoter}(\vec{T}; \mathcal{C}) \end{aligned} \quad (\text{S15})$$

in terms of the *Basal* and *Promoter* functions

$$\text{Basal}(\vec{T}; \mathcal{C}) = \frac{\sum_{|\vec{j}| \leq n} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} \left[\prod_{i=1}^{M_a} \left(\frac{\tilde{a}_i [T_i]}{K_i} \right)^{j_i} \right] \left[\prod_{i=M_a+1}^M \left(\frac{\tilde{r}_i [T_i]}{K_i} \right)^{j_i} \right]}{\sum_{|\vec{j}| \leq n} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} \left(\frac{[T_i]}{K_i} \right)^{j_i}}, \quad (\text{S16})$$

$$\text{Promoter}(\vec{T}; \mathcal{C}) = \frac{\sum_{\substack{|\vec{j}| \leq n \\ |\vec{j}_A| > 0}} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} \tilde{e}^{j_0} \left[\prod_{i=1}^{M_a} \left(\frac{\tilde{a}_i [T_i]}{K_i} \right)^{j_i} \right] \left[\prod_{i=M_a+1}^M \left(\frac{\tilde{r}_i [T_i]}{K_i} \right)^{j_i} \right]}{\sum_{|\vec{j}| \leq n} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} \left(\frac{[T_i]}{K_i} \right)^{j_i}}. \quad (\text{S17})$$

Let us note that, in [7], the particular expression of these functionals for modelling the transcriptional rates of two Shh target genes was deduced, by acting on an enhancer module with $n = 3$ sites, where $M = 3$ TFs can bind, $M_a = 2$ of them being activators and $M_r = 1$ repressor. In that work, the theoretical expressions assume the same affinity for activators and the repressor, and the activators have the same activation strength $\tilde{a}_1 = \tilde{a}_2 = 1$. Those expressions also consider total cooperativity between TFs and the effects of RNAP, and its affinity is involved in the constants r_{bas} and $v_{max}^{(n)}$.

Remark S1. A remarkable fact that can be pointed out from expressions (S12), (S13), (S15), (S16) and (S17) is that all the BEWARE operators, independently of the biochemical mechanisms involved, depend on the TF concentrations and their binding sites' affinities, $[T_i]$ and K_i , respectively, but

always through the quotients $[T_i]/K_i$. Despite being a trivial observation, this will be the key ingredient for the appearance of the elasticity when the variability of the activation/repression thresholds is analysed in terms of affinity variations.

These two approaches focus on the transcription process, which is the first mechanism involved in the genetic activity control. However, the whole process can be affected during the posterior RNA managing and interpretation. These processes in this work are assumed to be linear, and their effects are undercover in the value of the constants C_B , r_{bas} and $\nu_{max}^{(n)}$ appearing in (S12) and (S15).

S1.5. Simplification of BEWARE Operators' Expressions

One of the key points of this work is the fact that the regulation factor (S13), basal (S16) and promoter (S17) functions can be explicitly computed giving rise to simple rational and polynomial expressions whose analysis may contribute to the understanding of the general biological process. These calculations exploit a classical strategy employed for obtaining the derivation of the General Binding Equation more than a century ago [8]. For instance, in [9], the authors take advantage of particular cases of these simple expressions to deduce several transcription logics determined by the type of cooperativity between the TFs in the framework of Hh target genes. We start by remarking that the regulation factor, basal and promoter are rational functions where numerators and denominators correspond to polynomial expressions that can be expressed using the next definition.

Definition S1. Let $\vec{x} = (x_1, \dots, x_M) \in \mathbb{R}^M$, \mathcal{C} any of the cooperativity relations established in Subsections S1.1 (H2) and $C(\mathcal{C})$ determined by (S8)–(S10). Then, we define the polynomial function

$$S_e^{(n)}(\vec{x}; \mathcal{C}) = \sum_{|\vec{j}| \leq n} C(\mathcal{C}) \frac{n!}{\prod_{i=0}^M j_i!} e^{j_0} \prod_{i=1}^M x_i^{j_i}, \quad (\text{S18})$$

where the multi-index $\vec{j} \in \mathbb{N}_0^M$, $|\vec{j}| = \sum_{i=1}^M j_i$ and $j_0 = n - |\vec{j}|$.

Remark S2. Let us recall the vectorial notation for activators and repressors' concentrations $\vec{T}_A = ([T_1], \dots, [T_{M_a}]) \in \mathbb{R}^{M_a}$, $\vec{T}_R = ([T_{M_a+1}], \dots, [T_M]) \in \mathbb{R}^{M_r}$, the whole set of TF concentrations being $\vec{T} = (\vec{T}_A, \vec{T}_R) \in \mathbb{R}^M$. In concordance with previous vectors, let us also consider $\vec{a}, \vec{\tilde{a}} \in \mathbb{R}^{M_a}$ be the vector of the activation intensities, $a_i \geq 1$ and $\tilde{a}_i \leq 1$, $\forall i = 1, \dots, M_a$, $\vec{r}, \vec{\tilde{r}} \in \mathbb{R}^{M_r}$ be the vector of the repression intensities, $r_i, \tilde{r}_i \leq 1$ $\forall i = 1, \dots, M_r$, $\vec{K}_A \in \mathbb{R}_{M_a}$ be vector of the activators binding affinities, and $\vec{K}_R \in \mathbb{R}^{M_r}$ be the vector of the repressor binding affinities. Then, (S13), (S16), (S17) can be equivalently written as

$$F_{reg}(\vec{T}; \mathcal{C}) = \frac{S_1^{(n)}(\vec{a} \circ \vec{T}_A / \vec{K}_A, \vec{r} \circ \vec{T}_R / \vec{K}_R; \mathcal{C})}{S_1^{(n)}(\vec{T}_A / \vec{K}_A, \vec{T}_R / \vec{K}_R; \mathcal{C})}, \quad (\text{S19})$$

$$\text{Basal}(\vec{T}; \mathcal{C}) = \frac{S_1^{(n)}(\vec{\tilde{a}} \circ \vec{T}_A / \vec{K}_A, \vec{\tilde{r}} \circ \vec{T}_R / \vec{K}_R; \mathcal{C})}{S_1^{(n)}(\vec{T}_A / \vec{K}_A, \vec{T}_R / \vec{K}_R; \mathcal{C})}, \quad (\text{S20})$$

$$\begin{aligned} \text{Promoter}(\vec{T}; \mathcal{C}) &= \frac{S_e^{(n)}(\vec{\tilde{a}} \circ \vec{T}_A / \vec{K}_A, \vec{e}\vec{\tilde{r}} \circ \vec{T}_R / \vec{K}_R; \mathcal{C})}{S_1^{(n)}(\vec{T}_A / \vec{K}_A, \vec{T}_R / \vec{K}_R; \mathcal{C})} \\ &- \frac{S_e^{(n)}(\vec{0}, \vec{e}\vec{\tilde{r}} \circ \vec{T}_R / \vec{K}_R; \mathcal{C})}{S_1^{(n)}(\vec{T}_A / \vec{K}_A, \vec{T}_R / \vec{K}_R; \mathcal{C})} \end{aligned} \quad (\text{S21})$$

where $\vec{x} \circ \vec{y}$ and \vec{x}/\vec{y} denote the Hadamard (pointwise) product and division operators. Let us recall that the concatenation operator, (\vec{x}, \vec{y}) , is used to express the main argument of the previous expressions.

Our aim is to compute equivalent simplified expressions for (S18). In the case of non-cooperativity, $C(\mathcal{C}) = 1$, expression (S18) can be very easily computed,

$$S_e^{(n)}((x_1, \dots, x_M); \{T_1, \dots, T_d\}_1) = \left(e + \sum_{i=1}^M x_i \right)^n, \quad (\text{S22})$$

by direct application of the multinomial theorem. According to this result, we will adopt the mathematical definition of (S18) for empty vectors, that is, when $M = 0$, as $S_e^{(n)}(\cdot, \cdot) = e^n$.

On the other hand, in the presence of cooperativity, the multinomial theorem can not be applied so straightforwardly, and, in this case, the expression of (S18) is strictly determined by the cooperativity relations (S8)–(S9). However, as we state in the following proposition, we can use our definition of the general mixed cooperation (S10) and resume all the possible cooperative cases in one general expression of the function $S_e^{(n)}$.

Proposition S1. Let us assume $\mathcal{C} = \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}$ a general mixed cooperativity configuration, where \mathcal{I}_j denotes the indices of the vector x of the N subgroups of TFs cooperating with cooperativity constant c_j . Then,

$$S_e^{(n)}((x_1, \dots, x_M); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}) = \sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e + \sum_{j=1}^N h_j c_j \sum_{i \in \mathcal{I}_j} x_i \right)^n \prod_{j=1}^N \frac{(1 - \frac{1}{c_j})^{1-h_j}}{(c_j)^{h_j}} \quad (\text{S23})$$

where the addition on the multi-index $\vec{h} = (h_1, \dots, h_N) \in \mathbb{N}_0^N$ considers all the possible combinations where the components, h_j , can only be 0 or 1, that is, $|\vec{h}|_\infty = \max_j \{h_j\} \leq 1$.

In order to cover in this Proposition all the possible cooperative configurations, we first describe an iterative rule which will allow us to concrete the desired result for any mixed cooperativity configuration, covering as particular cases total and partial cooperativity.

Lemma S1. Let us assume a general mixed cooperativity configuration $\mathcal{C} = \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}$, where \mathcal{I}_i denotes the indices of the vector x of the N subgroups of TFs cooperating with cooperativity constant c_i . Let us consider \vec{x}_i a vector collecting all the values x_h with $h \in \mathcal{I}_i$. Then,

i) the reordering of the values x_h does not affect expression (S18), in particular,

$$S_e^{(n)}((x_1, \dots, x_M); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}) = S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_N); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}). \quad (\text{S24})$$

ii) The value of $S_e^{(n)}$ evaluated on N cooperating subfamilies of TFs admits a decomposition as the addition of two $S_e^{(n)}$ functions evaluated on $N - 1$ cooperative families, according to the next iterative rule:

$$\begin{aligned} & S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_{N-1}, \vec{x}_N); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_{c_N}\}) \\ &= \frac{1}{c_N} S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_{N-1}, c_N \vec{x}_N); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}) \\ &+ \left(1 - \frac{1}{c_N} \right) S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_{N-1}, \vec{0}); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}). \end{aligned}$$

Proof. Assertion i) is obviously true since the value of $S_e^{(n)}$ depends multiplicatively on x_i .

In order to prove *ii*), let us reorder the vector of indexes $\vec{j} = (\vec{j}_1, \dots, \vec{j}_N)$, $\vec{j}_N = (j_{\alpha+1}, \dots, j_M)$ being the $M - \alpha$ TFs cooperating with cooperativity c_N . By splitting the addition in terms those terms where \vec{j}_N is null and those where it is not, we can rewrite

$$\begin{aligned} S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_N); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}) \\ = \frac{1}{c_N} \sum_{\substack{|\vec{j}| \leq n \\ |\vec{j}_N| \geq 1}} \left(\prod_{i=1}^{N-1} c_i^{(|\vec{j}_i|-1)_+} \right) \frac{n!}{\prod_{i=0}^M j_i!} e^{j_0} \left(\prod_{i=1}^{\alpha} x_i^{j_i} \right) \left(\prod_{i=\alpha+1}^M (c_N x_i)^{j_i} \right) \\ + \sum_{\substack{|\vec{j}| \leq n \\ |\vec{j}_N| = 0}} \left(\prod_{i=1}^{N-1} c_i^{(|\vec{j}_i|-1)_+} \right) \frac{n!}{\prod_{i=0}^M j_i!} e^{j_0} \left(\prod_{i=1}^{\alpha} x_i^{j_i} \right). \end{aligned} \quad (\text{S25})$$

Then, adding and subtracting the term

$$\frac{1}{c_N} \sum_{\substack{|\vec{j}| \leq n \\ |\vec{j}_N| = 0}} \left(\prod_{i=1}^{N-1} c_i^{(|\vec{j}_i|-1)_+} \right) \frac{n!}{\prod_{i=0}^M j_i!} e^{j_0} \left(\prod_{i=1}^{\alpha} x_i^{j_i} \right) \left(\prod_{i=\alpha+1}^M (c_N x_i)^{j_i} \right),$$

in the previous expression, we obtain (S25)

$$\begin{aligned} S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_N); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_N\}_{c_N}\}) \\ = \frac{1}{c_N} \sum_{|\vec{j}| \leq n} \left(\prod_{i=1}^{N-1} c_i^{(|\vec{j}_i|-1)_+} \right) \frac{n!}{\prod_{i=0}^M j_i!} e^{j_0} \left(\prod_{i=1}^{\alpha} x_i^{j_i} \right) \left(\prod_{i=\alpha+1}^M (c_N x_i)^{j_i} \right) \\ + \left(1 - \frac{1}{c_N} \right) \sum_{\substack{|\vec{j}| \leq n \\ |\vec{j}_N| = 0}} \left(\prod_{i=1}^{N-1} c_i^{(|\vec{j}_i|-1)_+} \right) \frac{n!}{\prod_{i=0}^{\alpha} j_i!} e^{j_0} \left(\prod_{i=1}^{\alpha} x_i^{j_i} \right) \\ = \frac{1}{c_N} S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_{N-1}, c_N \vec{x}_N); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}) \\ + \left(1 - \frac{1}{c_N} \right) S_e^{(n)}((\vec{x}_1, \dots, \vec{x}_{N-1}, \vec{0}); \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}). \end{aligned}$$

□

The previous iterative property allows us to face up to the proof of Proposition S1.

Proof of Proposition S1. The proof of the formula stated can be done by induction over the number of cooperating subfamilies. As mentioned in the main text, expression (S23) reduces to (S27) in the case of a single cooperating family, that is, $N = 1$. (S27) can be easily deduced by applying S1 and taking into consideration (S22). Let us assume as inductive hypothesis that (S23) is valid when there exists $N - 1$ cooperative subfamilies. Then, by using twice Lemma S1, we can write:

$$\begin{aligned}
& S_e^{(n)}\left(\vec{x}_1, \dots, \vec{x}_{N-1}, \vec{x}_N; \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_{c_N}\}\right) \\
&= \frac{1}{c_N} S_e^{(n)}\left(\vec{x}_1, \dots, \vec{x}_{N-1}, c_N \vec{x}_N; \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}\right) \\
&+ \left(1 - \frac{1}{c_N}\right) S_e^{(n)}\left(\vec{x}_1, \dots, \vec{x}_{N-1}, \vec{0}; \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}\right) \\
&= \frac{S_e^{(n)}\left(\vec{x}_1, \dots, \vec{x}_{N-2}, c_{N-1} \vec{x}_{N-1}, c_N \vec{x}_N; \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-2}\}_{c_{N-2}}, \{\mathcal{T}_{N-1} \cup \mathcal{T}_N\}_1\}\right)}{c_N c_{N-1}} \\
&+ \left(1 - \frac{1}{c_{N-1}}\right) \frac{S_e^{(n)}\left(\vec{x}_1, \dots, \vec{x}_{N-2}, \vec{0}, c_N \vec{x}_N; \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-2}\}_{c_{N-2}}, \{\mathcal{T}_{N-1} \cup \mathcal{T}_N\}_1\}\right)}{c_N} \\
&+ \left(1 - \frac{1}{c_N}\right) S_e^{(n)}\left(\vec{x}_1, \dots, \vec{x}_{N-1}, \vec{0}; \{\{\mathcal{T}_1\}_{c_1}, \dots, \{\mathcal{T}_{N-1}\}_{c_{N-1}}, \{\mathcal{T}_N\}_1\}\right) \quad (S26)
\end{aligned}$$

Since all the $S_e^{(n)}$ operators are evaluated on data with only $N - 1$ cooperating subfamilies, we can apply to them the inductive hypothesis and observe that:

- the first term on the right-hand side of (S26) would correspond to all the additive terms on the right-hand side of (S23) with multi-indices of the form $(h_1, \dots, h_{N-2}, 1, 1)$;
- the second term on the right-hand side of (S26) would correspond to all the additive terms on the right-hand side (S23) with multi-indices of the form $(h_1, \dots, h_{N-2}, 0, 1)$;
- the third term on the right-hand side of (S26) would correspond to all the additive terms on the right-hand side (S23) with multi-indices of the form $(h_1, \dots, h_{N-2}, h_{N-1}, 0)$;

where all the h_j can be 0 or 1. Consequently, the addition of the three terms in (S26) coincides with the expression on the right-hand side of (S23), which concludes the proof. \square

Remark S3. From this general result, we can obtain, as particular cases, the value of (S18) in the total and partial cooperativity cases. In the total cooperativity case, all the TFs cooperate between them with the same cooperativity constant c , thus $N = 1$ and the possible values of \vec{h} are only $\vec{h} = (0)$ or $\vec{h} = (1)$, which leads to the expression

$$S_e^{(n)}((x_1, \dots, x_M); \{T_1, \dots, T_M\}_c) = \left(1 - \frac{1}{c}\right) e^n + \frac{1}{c} \left(e + c \sum_{i=1}^M x_i\right)^n. \quad (S27)$$

Let us observe that this expression coincides with (S22) when $c = 1$, that is, in the absence of cooperativity.

On the other side, if TFs cooperate only between the proteins of the same species, we have that $N = M$, $\mathcal{I}_i = \{i\}$, and expression (S23) reads

$$S_e^{(n)}((x_1, \dots, x_M); \{T_1\}_{c_1}, \dots, \{T_M\}_{c_M}) = \sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^M}} \left(e + \sum_{j=1}^M h_j c_j x_j\right)^n \prod_{j=1}^M \frac{\left(1 - \frac{1}{c_j}\right)^{1-h_j}}{(c_j)^{h_j}} \quad (S28)$$

for any M . Especially useful for the next section will be the case of only two TFs, $M = 2$. In this case, \vec{h} can be $(0, 0)$, $(1, 0)$, $(0, 1)$ or $(1, 1)$, giving rise to the expression:

$$\begin{aligned}
S_e^{(n)}((x_1, x_2); \{T_1\}_{c_1}, \{T_2\}_{c_2}) &= \left(1 - \frac{1}{c_1}\right) \left(1 - \frac{1}{c_2}\right) e^n \\
&+ \frac{(e + c_1 x_1)^n}{c_1} \left(1 - \frac{1}{c_2}\right) + \frac{(e + c_2 x_2)^n}{c_2} \left(1 - \frac{1}{c_1}\right) + \frac{(e + c_1 x_1 + c_2 x_2)^n}{c_1 c_2}. \quad (S29)
\end{aligned}$$

S2. Extreme Cooperativity Approach: Hill Modules

In this section, we are going to relate the developed thermodynamical modelling with a very frequently employed modelling approach in genetic control, the Hill modules. Using the analogy with Michaelis–Menten kinetic equations, some groups have proposed Michaelis-like functions for modelling genetic control (see [19,20] and references therein). This approach has been used in order to capture cooperativity effects via adding Hill coefficients [21] to the Michaelis-like functions. It is frequently assumed in the literature, see for instance [22–25], that the genetic transcription is controlled by combinations of Hill type functions. For example, the transcription obtained by a single activator/repressor corresponds to the expressions:

$$\frac{d[P]}{dt} = \alpha \frac{[A]^n}{K_d + [A]^n} + \eta \quad \frac{d[P]}{dt} = \frac{\beta}{K_d + [R]^n} + \gamma. \quad (\text{S30})$$

where α and β are proportional constants, and η , γ correspond respectively to optional basal or minimal expression levels.

In [19], the authors show that the Hill functions can approximate the transcription rates under conditions of high cooperativity. In this section, we will show that, indeed, the Hill functions (S30) can be framed into the thermodynamic approach by assuming extreme cooperativity.

The stimulated BEWARE operators corresponding to a single activator/repressor transcription factor would be obtained from (S15) and using

$$\text{Basal}([A]; \{A\}_c) = 1, \quad \text{Promoter}([A]; \{A\}_c) = \frac{\frac{1}{c} \left(\tilde{e} + c \frac{[A]}{K_A} \right)^n - \frac{1}{c} \tilde{e}^n}{\left(1 - \frac{1}{c} \right) + \frac{1}{c} \left(1 + c \frac{[A]}{K_A} \right)^n}, \quad (\text{S31})$$

in the single activator case

$$\text{Basal}([R]; \{R\}_c) = \frac{\left(1 - \frac{1}{c} \right) + \frac{1}{c} (1 + c \tilde{r}[R]/K_R)^n}{\left(1 - \frac{1}{c} \right) + \frac{1}{c} (1 + c[R]/K_R)^n}, \quad \text{Promoter}([R]; \{R\}_c) = 0 \quad (\text{S32})$$

for a single repressor. Let us remark that these expressions have been obtained from (S20), (S21) and Proposition S1.

The extreme cooperativity assumption implies that the cooperative binding constant c is large [19]. However, we cannot compute directly a limit in c without assuming some natural restrictions. By definition (see assumption H2 in Section S1.1), the cooperativity interactions essentially modulate the binding affinity of the transcription factors in the binding sites. More specifically, if K_* is the affinity constant of a transcription factor, the affinity constant of a consecutive cleavage will be K_*/c with $c > 1$ (recall the inverse relation where, for lower affinity constants, the transcription factor will have higher binding affinity). Moreover, if we fill the n enhancers with the same transcription factor, it is clear to see that the “global” affinity constant of the whole process will be $K_d = K_*^n/c^{n-1}$. The extreme cooperativity assumption claims that, since we are interested in only modifying the cooperativity between the transcription factors, we will compute a limit of $c \rightarrow \infty$ without modifying the global affinity constant in the process, keeping $K_d = cte$ (and, consequently, $K_* \rightarrow 0$ [19]). These limits can be computed easily, so we obtain

$$\begin{aligned} \lim_{\substack{c \rightarrow \infty \\ K_d = cte}} \text{Promoter}([A]; \{A\}_c) &= \frac{[A]^n}{K_d + [A]^n}, \\ \lim_{\substack{c \rightarrow \infty \\ K_d = cte}} \text{Basal}([R]; \{R\}_c) &= \frac{K_d + \tilde{r}^n [R]^n}{K_d + [R]^n} \end{aligned}$$

which allow us to justify previous Hill modules because the limits of the corresponding BEWARE operators coincide with (S30) by taking $\alpha = \frac{r_{bas}}{1+K_{RP}[RNAP]^{-1}}$, $\eta = \frac{v_{max}^{(n)}}{1+K_{RP}[RNAP]^{-1}}$, $\beta = \frac{r_{bas}K_d(1-\tilde{r}^n)}{1+K_{RP}[RNAP]^{-1}}$ and $\gamma = \frac{r_{bas}\tilde{r}^n}{1+K_{RP}[RNAP]^{-1}}$. Let us notice that the optimal values η and γ vanish when $r_{bas} = 0$ or $\tilde{r} = 0$, respectively, which corresponds in the thermodynamical model to null basal level and a repressor executing a total repression once it is bound. Indeed, these are some extra hypotheses employed in [19] for justifying (S30) (with $\eta = 0$ and $\gamma = 0$) that are not required in our approach.

It is also interesting to mention that the same reasoning can be used to prove that, under the extreme cooperativity assumption, the recruitment BEWARE operators also converge toward a generalised Hill type functions. Since

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} F_{reg}([A]; \{A\}_c) = \frac{K_d + a^n[A]^n}{K_d + [A]^n},$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} F_{reg}([R]; \{R\}_c) = \frac{K_d + r^n[R]^n}{K_d + [R]^n},$$

the associated recruitment BEWARE operators verify

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} \text{BEWARE}_r([A], [RNAP]; \{A\}_c) = \alpha \frac{[A]^n}{\delta K_d + [A]^n} + \eta,$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} \text{BEWARE}_r([R], [RNAP]; \{R\}_c) = \frac{\beta}{\epsilon K_d + [R]^n} + \gamma,$$

where now $\eta = \frac{C_B}{1 + \frac{K_{RP}}{[RNAP]}}$ and $\gamma = \frac{C_B r^n}{r^n + \frac{K_{RP}}{[RNAP]}}$ are respectively a basal and minimal transcrip-

tional rates, and $\alpha = \frac{C_B}{1 + \frac{K_{RP}}{[RNAP]}} \frac{a^n - 1}{a^n + 1}$, $\beta = \frac{K_{RP}}{[RNAP]} \frac{C_B K_d (1 - r^n)}{(r^n + \frac{K_{RP}}{[RNAP]})^2}$, $\delta = \frac{1 + \frac{K_{RP}}{[RNAP]}}{a^n + \frac{K_{RP}}{[RNAP]}}$ and $\epsilon = \frac{1 + \frac{K_{RP}}{[RNAP]}}{r^n + \frac{K_{RP}}{[RNAP]}}$ are constants. Let us observe that the limits in this case are generalisations of the Hill classical modules (S30) because of parameters $\delta < 1$ and $\epsilon > 1$.

When the modelling by Hill modules involves the effects of several transcription factors (see, for instance, [23]), it is not so clear what the counterparts of (S30) are. If the binding sites or any TF are independent and there are no cooperativity interactions, the Hill candidates can be computed straightforwardly [6,22]. However, to the authors' best knowledge, the same question is not clear when the TFs compete for the same binding sites or cooperate between them. Our modelling approach gives us a clear strategy to propose Hill type modules in the presence of several TFs competing for the same enhancers. They might be deduced from the extreme cooperativity limit of the stimulated/recruitment BEWARE operators if the total cooperativity holds between the TFs:

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} F_{reg}([T_1], \dots, [T_M]; \{\{T_1, \dots, T_M\}_c\}) = \frac{K_d + \left(\sum_{i=1}^M a_i [T_i]\right)^n}{K_d + \left(\sum_{i=1}^M [T_i]\right)^n}, \quad (\text{S33})$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} \text{Basal}([T_1], \dots, [T_M]; \{\{T_1, \dots, T_M\}_c\}) = \frac{K_d + \left(\sum_{i=1}^M \tilde{a}_i [T_i]\right)^n}{K_d + \left(\sum_{i=1}^M [T_i]\right)^n}, \quad (\text{S34})$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Promoter([T_1], \dots, [T_M]; \{\{T_1, \dots, T_M\}_c\}) \\ = \frac{\left(\sum_{i=1}^{M_d} \tilde{a}_i [T_i] + \sum_{j=M_d+1}^M \tilde{e} r_i [T_j]\right)^n - \left(\sum_{j=M_d+1}^M \tilde{e} r_i [T_j]\right)^n}{K_d + \left(\sum_{i=1}^M [T_i]\right)^n},$$

or

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Freg([T_1], \dots, [T_M]; \{\{T_1\}_c, \dots, \{T_M\}_c\}) = \frac{K_d + \sum_{i=1}^M (a_i [T_i])^n}{K_d + \sum_{i=1}^M [T_i]^n}, \quad (S35)$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Basal([T_1], \dots, [T_M]; \{\{T_1\}_c, \dots, \{T_M\}_c\}) = \frac{K_d + \sum_{i=1}^M (\tilde{a}_i [T_i])^n}{K_d + \sum_{i=1}^M [T_i]^n}, \quad (S36)$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Promoter([T_1], \dots, [T_M]; \{\{T_1\}_c, \dots, \{T_M\}_c\}) = \frac{\sum_{i=1}^{M_d} (\tilde{a}_i [T_i])^n}{K_d + \sum_{i=1}^M [T_i]^n},$$

when only partial cooperativity is present between TFs. In order to deduce expressions (S34)–(S37), we need to recall first the general expressions of the Regulation Factor, Basal and Promoter functions defined in Remark S2 in terms of the polynomial function (S18). Since all these functions are written in terms of rational relations between S_e , we can define a general rational function

$$R^{(n)} = \frac{S_{e_1}^{(n)}((x_1/K, \dots, x_M/K); \{\{\mathcal{X}_1\}_c, \dots, \{\mathcal{X}_N\}_c\})}{S_{e_2}^{(n)}((y_1/K, \dots, y_M/K); \{\{\mathcal{Y}_1\}_c, \dots, \{\mathcal{Y}_N\}_c\})} \quad (S37)$$

with $S_e^{(n)}$ defined in the mixed cooperative case (S23). Then, if we define the global affinity constant $K_d = K^n / c^{n-1}$, we can rewrite (S37)

$$R^{(n)} = \frac{\sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e_1 + \sum_{j=1}^N h_j c \sum_{i \in \mathcal{I}_j} \frac{x_i}{K} \right)^n \prod_{j=1}^N \frac{(1 - \frac{1}{c})^{1-h_j}}{c^{h_j}} \sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e_1 + \frac{c}{K} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} x_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}}{\sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e_2 + \sum_{j=1}^N h_j c \sum_{i \in \mathcal{I}_j} \frac{y_i}{K} \right)^n \prod_{j=1}^N \frac{(1 - \frac{1}{c})^{1-h_j}}{c^{h_j}} \sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e_2 + \frac{c}{K} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} y_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}} \\ = \frac{\sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e_1 + \frac{c^{1/n}}{K_d^{1/n}} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} x_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}}{\sum_{\substack{|\vec{h}|_\infty \leq 1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(e_2 + \frac{c^{1/n}}{K_d^{1/n}} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} y_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}}.$$

By splitting the sums in those terms that have $|\vec{h}|_\infty = 0$ and $|\vec{h}|_\infty = 1$, we finally obtain

$$R^{(n)} = \frac{(c-1)^N + \frac{c}{K_d} \sum_{\substack{|\vec{h}|_\infty=1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(\frac{K_d^{1/n}}{c^{1/n}} e_1 + \frac{c^{1/n}}{K_d^{1/n}} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} x_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}}{(c-1)^N + \frac{c}{K_d} \sum_{\substack{|\vec{h}|_\infty=1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(\frac{K_d^{1/n}}{c^{1/n}} e_2 + \frac{c^{1/n}}{K_d^{1/n}} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} y_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}}.$$

Please note that both $(c-1)^N$ and $\frac{c}{K_d} \sum_{\substack{|\vec{h}|_\infty=1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(\frac{K_d^{1/n}}{c^{1/n}} e_1 + \frac{c^{1/n}}{K_d^{1/n}} \sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} x_i \right)^n \prod_{j=1}^N (c-1)^{1-h_j}$ are

polynomials of degree N in the c variable, since $|\vec{h}|_\infty = 1$ automatically implies that $\sum_{j=1}^N 1 - h_j \leq N - 1$. We are interested in computing $\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} R^{(n)}$; hence, by l'Hôpital's rule,

the only terms that will remain in the limit will be those coefficients that multiply to c^N , that is, those terms such as $|\vec{h}| = 1$:

$$\begin{aligned} \lim_{\substack{c \rightarrow \infty \\ K_d = cte}} R^{(n)} &= \frac{1 + \frac{1}{K_d} \sum_{\substack{|\vec{h}|=1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(\sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} x_i \right)^n}{1 + \frac{1}{K_d} \sum_{\substack{|\vec{h}|=1 \\ \vec{h} \in \mathbb{N}_0^N}} \left(\sum_{j=1}^N h_j \sum_{i \in \mathcal{I}_j} y_i \right)^n} = \frac{K_d + \sum_{j=1}^N \left(\sum_{i \in \mathcal{I}_j} x_i \right)^n}{K_d + \sum_{j=1}^N \left(\sum_{i \in \mathcal{I}_j} y_i \right)^n}. \end{aligned} \quad (\text{S38})$$

Please note that we can now directly apply the definitions of the Remark S2 and deduce automatically the expressions (S34)-(S37) assuming that $K_i = K$ and $c_i = c$ for all $i = 1, \dots, M$.

S3. Global Activator/Repressor Variables Reduction

In [3], the problem of collecting the control executed by different TFs into two global Activation/Repression variables in the particular case of the Shh system was addressed. In these lines, we reconsider the same question and, according to the modelling developed in the previous section, we describe when BEWARE operators are really controlled by global Activator/Repressor variables and how to define those variables in terms of the multiple TFs involved in the system. Actually, we can make the next remark.

Remark S4. *The effect in the BEWARE operator of transcription factors*

- with the same signal strength (same transcriptional intensity/rate) in the recruitment/stimulated approaches;
- and cooperating in the same subset;

can be summarised in a unique single variable, even if they have different affinities.

For instance, let us take into consideration the previous Remark in the stimulated BEWARE operator deduced in [7,18] for some Shh target genes. The transcription is controlled by the concentration balance of $M = 3$ TFs: a pair of activators *GliA*, *Gli3A* and a repressor *Gli3R* competing for $n = 3$ enhancers. Gli-DNA enhancers' binding affinities were described by dissociation constants K_1 for *Gli1* and K_3 for both forms of *Gli3*. Since the model suggested total cooperativity between all the TFs and the activators, *GliA* and

Gli3A, it can be seen that the BEWARE operator deduced in [7,18] depends on the global activator variable

$$[A] = \frac{[GliA]}{\bar{K}_1} + \frac{[Gli3A]}{\bar{K}_3}$$

with dissociation constant $K_A = \frac{K_1 + K_3}{2}$, where the weights for defining A are given by $\bar{K}_1 = \frac{K_1}{K_A}$, $\bar{K}_3 = \frac{K_3}{K_A}$. Since only one repressor was taken into consideration, *Gli3R*, this plays the role of repressor variable with dissociation constant K_3 . The fact that the BEWARE operators only depend on these two global variables can be easily checked by replacing the new variables in the expressions of $S_{\bar{e}}$ of (S19), (S20) and (S21), and noting that the new functions obtained correspond to the expressions of the functions $S_{\bar{e}}$ in the case of $M = 2$ transcription factors.

Remark S5. Generalising the previous example, we always have that:

- all the activators (repressors) have the same signal strength;
- and all the activators (repressors) cooperate in the same family with the same cooperativity constant;

then, their effect in the BEWARE functional can be summarised in global activator (repressor) variables:

$$[A] = \sum_{i=1}^{M_a} \frac{[T_i]}{\bar{K}_i}; \bar{K}_i = \frac{K_i}{K_A} \text{ being } K_A = \frac{\sum_{i=1}^{M_a} K_i}{M_a},$$

$$[R] = \sum_{i=M_a+1}^M \frac{[T_i]}{\bar{K}_i}; \bar{K}_i = \frac{K_i}{K_R} \text{ being } K_R = \frac{\sum_{i=M_a+1}^M K_i}{M_r}.$$

Anyway, the BEWARE functionals we are going to consider in the next paragraphs are those defined by (S12) or (S15), in correspondence with the recruitment/stimulated approaches, where only two functional opposite transcription factors are considered: an activator, A, and a repressor R. Indeed, these are the BEWARE functionals we have introduced in the main manuscript for the sake of clarity. In that case, only two admissible cooperative relations are possible: $\{\{A, R\}_c\}$ or $\{\{A\}_{c_A}, \{R\}_{c_R}\}$, corresponding to the total and partial cooperativity cases. Then, by Remark S2 and Proposition S1, we obtain that the regulation factor, basal and promoter functions read

$$F_{reg}([A], [R]; \{\{A, R\}_c\}) = \frac{\left(1 - \frac{1}{c}\right) + \frac{1}{c}(1 + ca[A]/K_A + cr[R]/K_R)^n}{\left(1 - \frac{1}{c}\right) + \frac{1}{c}(1 + c[A]/K_A + c[R]/K_R)^n}, \quad (S39)$$

$$Basal([A], [R]; \{\{A, R\}_c\}) = \frac{\left(1 - \frac{1}{c}\right) + \frac{1}{c}(1 + c[A]/K_A + c[R]/K_R)^n}{\left(1 - \frac{1}{c}\right) + \frac{1}{c}(1 + c[A]/K_A + c[R]/K_R)^n}, \quad (S40)$$

$$Promoter([A], [R]; \{\{A, R\}_c\}) = \frac{\frac{1}{c}(\tilde{e} + c\frac{[A]}{K_A} + c\tilde{e}\tilde{r}\frac{[R]}{K_R})^n - \frac{1}{c}(\tilde{e} + c\tilde{e}\tilde{r}\frac{[R]}{K_R})^n}{\left(1 - \frac{1}{c}\right) + \frac{1}{c}(1 + c\frac{[A]}{K_A} + c\frac{[R]}{K_R})^n}, \quad (S41)$$

for the total cooperativity case and look like

$$F_{reg}([A], [R]; \{\{A\}_{c_A}, \{R\}_{c_R}\}) \quad (S42)$$

$$= \frac{\left(1 - \frac{1}{c_A}\right)\left(1 - \frac{1}{c_R}\right) + \frac{\left(1 - \frac{1}{c_R}\right)}{c_A}\left(1 + \frac{ac_A[A]}{K_A}\right)^n + \frac{\left(1 - \frac{1}{c_A}\right)}{c_R}\left(1 + \frac{rc_R[R]}{K_R}\right)^n + \frac{\left(1 + \frac{ac_A[A]}{K_A} + \frac{rc_R[R]}{K_R}\right)^n}{c_A c_R}}{\left(1 - \frac{1}{c_A}\right)\left(1 - \frac{1}{c_R}\right) + \frac{\left(1 - \frac{1}{c_R}\right)}{c_A}\left(1 + \frac{c_A[A]}{K_A}\right)^n + \frac{\left(1 - \frac{1}{c_A}\right)}{c_R}\left(1 + \frac{c_R[R]}{K_R}\right)^n + \frac{\left(1 + \frac{c_A[A]}{K_A} + \frac{c_R[R]}{K_R}\right)^n}{c_A c_R}},$$

$$\text{Basal}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\} \quad (\text{S43})$$

$$= \frac{\left(1 - \frac{1}{c_A}\right)\left(1 - \frac{1}{c_R}\right) + \frac{\left(1 - \frac{1}{c_R}\right)}{c_A} \left(1 + \frac{c_A[A]}{K_A}\right)^n + \frac{\left(1 - \frac{1}{c_A}\right)}{c_R} \left(1 + \frac{c_R[R]}{K_R}\right)^n + \frac{\left(1 + \frac{c_A[A]}{K_A} + \frac{c_R[R]}{K_R}\right)^n}{c_A c_R}}{\left(1 - \frac{1}{c_A}\right)\left(1 - \frac{1}{c_R}\right) + \frac{\left(1 - \frac{1}{c_R}\right)}{c_A} \left(1 + \frac{c_A[A]}{K_A}\right)^n + \frac{\left(1 - \frac{1}{c_A}\right)}{c_R} \left(1 + \frac{c_R[R]}{K_R}\right)^n + \frac{\left(1 + \frac{c_A[A]}{K_A} + \frac{c_R[R]}{K_R}\right)^n}{c_A c_R}} \quad (\text{S44})$$

$$\text{Promoter}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\} \quad (\text{S45})$$

$$= \frac{\frac{\left(1 - \frac{1}{c_R}\right)}{c_A} \left(\tilde{e} + \frac{c_A[A]}{K_A}\right)^n + \frac{\left(\tilde{e} + \frac{c_A[A]}{K_A} + \frac{\tilde{e} c_R[R]}{K_R}\right)^n}{c_A c_R} - \frac{\left(1 - \frac{1}{c_R}\right)}{c_A} \tilde{e}^n - \frac{\left(\tilde{e} + \frac{\tilde{e} c_R[R]}{K_R}\right)^n}{c_A c_R}}{\left(1 - \frac{1}{c_A}\right)\left(1 - \frac{1}{c_R}\right) + \frac{\left(1 - \frac{1}{c_R}\right)}{c_A} \left(1 + \frac{c_A[A]}{K_A}\right)^n + \frac{\left(1 - \frac{1}{c_A}\right)}{c_R} \left(1 + \frac{c_R[R]}{K_R}\right)^n + \frac{\left(1 + \frac{c_A[A]}{K_A} + \frac{c_R[R]}{K_R}\right)^n}{c_A c_R}} \quad (\text{S46})$$

in the case of partial cooperativity.

Let us observe that these expressions allow us to relate the parameters in these models with measurable reference values as the minimal, basal and maximal expression levels, following the ideas presented in [3]. These values, collected in Table S1, can be easily computed by letting $[A] \rightarrow 0$, $[R] \rightarrow \infty$ or $[A] \rightarrow 0$, $[R] \rightarrow 0$ or $[A] \rightarrow \infty$, $[R] \rightarrow 0$, respectively. It is important to note that this result also works in the extreme cooperative framework (i.e., Hill operators), since the levels in Table S1 do not depend on the TFs' cooperativity.

Table S1. Theoretical values for minimal, basal and maximal transcriptional rates for BEWARE operators in the presence of global activator/repressor variables.

	Minimal	Basal	Maximal
Recruitment	$\frac{C_B}{1 + \frac{K_{RP}}{[RNAP]^n}}$	$\frac{C_B}{1 + \frac{K_{RP}}{[RNAP]}}$	$\frac{C_B}{1 + \frac{K_{RP}}{[RNAP]^n}}$
Stimulated	$\frac{r_{bas} \tilde{e}^n}{1 + \frac{K_{RP}}{[RNAP]}}$	$\frac{r_{bas}}{1 + \frac{K_{RP}}{[RNAP]}}$	$\frac{r_{bas} + \nu_{max}^{(n)}}{1 + \frac{K_{RP}}{[RNAP]}}$

S4. Existence/Non Existence of Inverse Logic in the Activator/Repressor Framework: Pull Effect

In this Section, we are going to analyse the consistency of previous expressions with the fundamental notion of activators established in [26], that is, when activator concentrations are scaled up, then the transcriptional activity increases. Analogously, it can be declared the fundamental notion of repressors saying that increases in repressor concentrations would diminish transcriptional activity. This basic idea is translated mathematically into:

$$\frac{\partial \text{BEWARE}([A], [R], [RNAP]; C)}{\partial [A]} > 0, \quad (\text{S46})$$

$$\frac{\partial \text{BEWARE}([A], [R], [RNAP]; C)}{\partial [R]} < 0. \quad (\text{S47})$$

However, as we will show, this basic logic may not hold in some of the proposed models, and effects of 'inverse control logic' (i.e., an inversion on the inequalities (S46) and (S47)) can appear. In this case, both versions of modelling stimulated and recruitment coincide, predicting that this inverse logic would happen in the presence of strong enough cooperativity between activators and repressors. Furthermore, they coincide predicting that, in the presence of cooperativity only between TFs of the same nature, that is partial cooperativity, the basic activator/repressor logic (S46)–(S47) always holds.

The results of the performed analysis are summarised in the next Lemma.

Lemma S2. BEWARE operators (S12)–(S15) depending only on two functional opposite TFs

- i) can exhibit inverse control logic when total cooperativity is considered. This occurs for large enough cooperativities for certain ranges of the parameters a , r , $v_{max}^{(n)}$ and r_{bas} established in Table S2;
- ii) However, this basic control logic always holds when partial cooperativity (S9) is considered.

Table S2. Parameter requirements for the existence of inverse logic for bifunctional BEWARE operators in the presence of total cooperativity.

	Act. Inv. Log. (S46)	Rep Inv. Log. (S47)
Recruitment	$r < a^{\frac{1}{1-n}} \ \& \ c > c_{th}^a$	$r > a^{1-n} \ \& \ c > c_{th}^r$
Stimulated	$\frac{v_{max}^{(n)}}{r_{bas}} < \frac{1-\tilde{r}^{n-1}}{(\tilde{e}\tilde{r})^{n-1}} \ \& \ c > \tilde{c}_{th}^a$	$\frac{r_{bas}}{v_{max}^{(n)}} < \frac{\tilde{e}\tilde{r}}{1-\tilde{r}} \ \& \ c > \tilde{c}_{th}^r$

The analysis developed in this Section for proving Lemma S2 also allows for describing the values of the concentrations that give rise to inverse logic. In the presence of relative low concentrations of activators (that is, low activators' concentrations compared to the concentrations of repressors), the system could show an unnatural response to an increase of the activators, presenting a decrement in the transcription rate (BEWARE function). This 'pull effect' is a direct consequence of the total cooperativity. Since an increase of the activator concentration implies more activator bindings, these additional bindings improve the cleavage of any other transcription factor, including repressors. Taking into account that there is a higher concentration of repressors in the system than activators, it is much more likely to 'pull' repressors in the enhancers, finding as a consequence more states with more repressors than activators (i.e., more repression and hence less transcription). The delicate balances occurring in that case between activation, repression, affinities and binding cooperativity are reflected in Table S2. Figure S1 illustrates these effects. Although behavioural tendencies (S46)–(S47) could appear to be very naive, their relevance in our logical scheme is undoubtable. This can be seen in the proof of Proposition S2 where the existence of activation/repression thresholds is stated. In those cases where (S46)–(S47) does not hold, this requires an extra effort in order to apply the Implicit Function Theorem. These kinds of basic properties are also really relevant in specific applications, as for instance in repressilator type models [27].

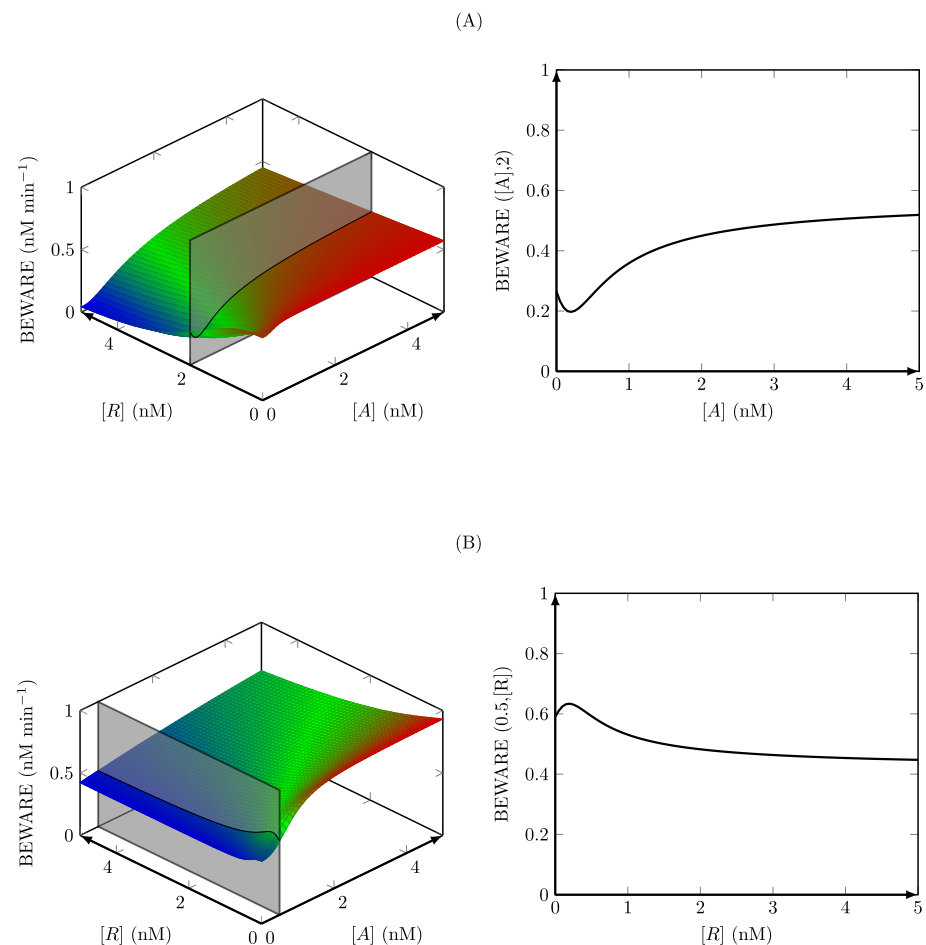


Figure S1. Inverse logic for a Recruitment BEWARE operator with total cooperativity. (A) activators' inverse logic; (B) repressors' inverse logic

In these arguments, it will be helpful to use the positive symmetric functions defined by:

$$H_n(X, Y) = \sum_{i=0}^{n-1} X^i Y^{n-1-i} \text{ for any } X, Y \in (0, +\infty) \quad (\text{S48})$$

when $n > 1$ and $H_1(X, Y) = 1$ when $n = 1$.

It can be easily checked that, using this notation, we have

$$X^{n-1} - Y^{n-1} = H_{n-1}(X, Y)(X - Y) = \frac{H_{n-1}(X, Y)}{H_n(X, Y)}(X^n - Y^n) \quad n \geq 2. \quad (\text{S49})$$

We will employ subsequently several easy properties of these functions collected in the next Lemma.

Lemma S3. Letting $X, Y \in (0, +\infty)$, then

1. $\frac{H_{n-1}(X, Y)}{H_n(X, Y)}$ is strictly decreasing with respect to X and Y .
2. If $X > Y$, the following relations are fulfilled:

$$\frac{1}{\frac{Y}{n-1} + X} \leq \frac{H_{n-1}(X, Y)}{H_n(X, Y)} \leq \frac{1}{\frac{X}{n-1} + Y}.$$

3. If $Z \leq X$, then $\frac{H_{n-1}(X, Y)}{H_n(X, Y)} Z < 1$.

Proof. Properties *i*) and *ii*) can be trivially proven by using the identities:

$$\frac{H_{n-1}(X, Y)}{H_n(X, Y)} = \frac{1}{\frac{X}{\sum_{i=0}^{n-2} (Y/X)^i} + Y} = \frac{1}{\frac{Y}{\sum_{i=0}^{n-2} (X/Y)^i} + X}.$$

Estimating *iii*) can be also checked by considering

$$\frac{H_{n-1}(X, Y)}{H_n(X, Y)} Z = \frac{\sum_{i=0}^{n-2} Y^i X^{n-2-i} Z}{\sum_{i=0}^{n-1} Y^i X^{n-1-i}} \leq \frac{\sum_{i=0}^{n-2} Y^i X^{n-1-i}}{\sum_{i=0}^{n-1} Y^i X^{n-1-i}} < 1.$$

□

S4.1. Existence of Inverse Logic in the Presence of High Total Cooperativity

This subsection is devoted to prove statement *i*) in Lemma S2. That is, in the presence of strong enough total cooperativity, recruitment and stimulated approaches predict that the intuitive behavioural tendencies (S46) or (S47) can be violated. For the sake of simplicity, we will note $\tilde{x} = \frac{c[A]}{K_A}$ and $\tilde{y} = \frac{c[R]}{K_R}$.

- Activators inverse logic for the Recruitment BEWARE operator
Since the recruitment BEWARE operator is increasing with respect to the regulation factor operator, we have

$$\begin{aligned} & \text{sign} \left\{ \frac{\partial \text{BEWARE}_r([A], [R], [RNAP]; \{A, R\}_c)}{\partial [A]} \right\} \\ &= \text{sign} \left\{ \frac{\partial F_{reg}(\tilde{x}, \tilde{y}); \{A, R\}_c}{\partial \tilde{x}} \right\} \\ &= \text{sign} \left\{ (c-1)(a\bar{\alpha}_r^{n-1} - \alpha_r^{n-1}) + \bar{\alpha}_r^{n-1} \alpha_r^{n-1} (a\alpha_r - \bar{\alpha}_r) \right\} \end{aligned} \quad (\text{S50})$$

where $\bar{\alpha}_r = \bar{\alpha}_r(\tilde{x}, \tilde{y}) = 1 + a\tilde{x} + r\tilde{y}$, $\alpha_r = \alpha_r(\tilde{x}, \tilde{y}) = 1 + \tilde{x} + \tilde{y}$. Since we are assuming $r < 1 < a$, we have that $a\alpha_r - \bar{\alpha}_r > 0$. This proves that, in the absence of cooperativity ($c = 1$), the sign of (S50) will always be positive. Nevertheless, if $a\bar{\alpha}_r^{n-1} - \alpha_r^{n-1}$ takes a negative value and c is high enough, that is,

$$c > 1 + \frac{\bar{\alpha}_r^{n-1} \alpha_r^{n-1} (a\alpha_r - \bar{\alpha}_r)}{\alpha_r^{n-1} - a\bar{\alpha}_r^{n-1}} \quad (\text{S51})$$

the sign of (S50) can become negative. It can be checked that $(a\bar{\alpha}_r^{n-1} - \alpha_r^{n-1}) < 0$ if and only if $a^{\frac{1}{n-1}} r < 1$ and, in that case, it would occur for values in the cone

$$H_{r,a} = \left\{ (\tilde{x}, \tilde{y}) \in (\mathbf{R}_0^+)^2; \tilde{y} > \frac{(a^{\frac{1}{n-1}} - 1) + (a^{\frac{n}{n-1}} - 1)\tilde{x}}{1 - a^{\frac{1}{n-1}} r} \right\}.$$

Thus, for any

$$c > c_{th}^a = \inf_{(\tilde{x}, \tilde{y}) \in H_{r,a}} \left\{ 1 + \frac{\bar{\alpha}_r^{n-1} \alpha_r^{n-1} (a\alpha_r - \bar{\alpha}_r)}{\alpha_r^{n-1} - a\bar{\alpha}_r^{n-1}} \right\},$$

we can assure that there exist some values $(\tilde{x}, \tilde{y}) \in H$ such that the sign computed in (S50) will be negative.

- Repressors inverse logic for the Recruitment BEWARE operator
By an analogous argument, we obtain

$$\begin{aligned} & \text{sign} \left\{ \frac{\partial \text{BEWARE}_r([A], [R], [\text{RNAP}]; \{A, R\}_c)}{\partial [R]} \right\} \\ &= \text{sign} \left\{ \frac{\partial F_{reg}(\tilde{x}, \tilde{y})}{\partial \tilde{y}} \right\} \\ &= \text{sign} \left\{ (c-1)(r\tilde{\alpha}_r^{n-1} - \alpha_r^{n-1}) + \tilde{\alpha}_r^{n-1} \alpha_r^{n-1} (r\alpha_r - \tilde{\alpha}_r) \right\} \end{aligned} \quad (\text{S52})$$

Since we are assuming $r < 1 < a$, we now have that $r\alpha_r - \tilde{\alpha}_r < 0$. Thus, the sign of (S52) will be always negative in the absence of cooperativity $c = 1$. On the other hand, if $(r\tilde{\alpha}_r^{n-1} - \alpha_r^{n-1})$ takes a positive value and c is high enough, that is,

$$c > 1 + \frac{\tilde{\alpha}_r^{n-1} \alpha_r^{n-1} (\tilde{\alpha}_r - r\alpha_r)}{r\tilde{\alpha}_r^{n-1} - \alpha_r^{n-1}} \quad (\text{S53})$$

the sign of (S52) can become positive. It can be checked that $(r\tilde{\alpha}_r^{n-1} - \alpha_r^{n-1}) > 0$ if and only if $a r^{\frac{1}{n-1}} > 1$ and, in that case, it would happen for values in the cone

$$H_{r,r} = \left\{ (\tilde{x}, \tilde{y}) \in (\mathbf{R}_0^+)^2; \tilde{x} > \frac{(1 - r^{\frac{1}{n-1}}) + (1 - r^{\frac{n}{n-1}})\tilde{y}}{a r^{\frac{1}{n-1}} - 1} \right\}.$$

Thus, for any

$$c > c_{th}^r = \inf_{(\tilde{x}, \tilde{y}) \in H_{r,r}} \left\{ 1 + \frac{\tilde{\alpha}_r^{n-1} \alpha_r^{n-1} (\tilde{\alpha}_r - r\alpha_r)}{r\tilde{\alpha}_r^{n-1} - \alpha_r^{n-1}} \right\},$$

we can assure that there exist some values $(\tilde{x}, \tilde{y}) \in H$ such that the sign computed in (S52) will be positive.

- Activators Inverse Logic for the Stimulated BEWARE Operator

$$\begin{aligned} & \text{sign} \left\{ \frac{\partial \text{BEWARE}_s([A], [R], [\text{RNAP}]; \{A, R\}_c)}{\partial [A]} \right\} \\ &= \text{sign} \left\{ \frac{r_{bas}}{1 + \frac{K_{RP}}{[\text{RNAP}]}} \frac{\partial \text{Basal}(\tilde{x}, \tilde{y})}{\partial \tilde{x}} + \frac{v_{max}^{(n)}}{1 + \frac{K_{RP}}{[\text{RNAP}]}} \frac{\partial \text{Promoter}(\tilde{x}, \tilde{y})}{\partial \tilde{x}} \right\} \\ &= \text{sign} \left\{ (c-1) \left((\tilde{\alpha}_s^{n-1} - \alpha_s^{n-1}) r_{bas} + \beta_s^{n-1} v_{max}^{(n)} \right) \right. \\ &\quad \left. + \alpha_s^{n-1} \left(\tilde{\alpha}_s^{n-1} (\alpha_s - \tilde{\alpha}_s) r_{bas} + (\beta_s^{n-1} (\alpha_s - \beta_s) + \tilde{\beta}_s) v_{max}^{(n)} \right) \right\} \end{aligned} \quad (\text{S54})$$

where, adopting the previous notation, we have $\tilde{\alpha}_s = \tilde{\alpha}_s(\tilde{x}, \tilde{y}) = 1 + \tilde{x} + \tilde{r}\tilde{y}$, $\alpha_s = \alpha_s(\tilde{x}, \tilde{y}) = 1 + \tilde{x} + \tilde{y}$, $\tilde{\beta}_s = \tilde{\beta}_s(\tilde{y}) = \tilde{e} + \tilde{e}\tilde{r}\tilde{y}$, $\beta_s = \beta_s(\tilde{x}, \tilde{y}) = \tilde{e} + \tilde{x} + \tilde{e}\tilde{r}\tilde{y}$. Please note also that, thanks to $\tilde{r} < 1$ and $X, Y \geq 0$, the next relations are verified

$$\alpha_s \geq \tilde{\alpha}_s \geq \beta_s \geq \tilde{\beta}_s > 0. \quad (\text{S55})$$

By using these estimates, we can easily check

$$\alpha_s^{n-1} \left(\tilde{\alpha}_s^{n-1} (\alpha_s - \tilde{\alpha}_s) r_{bas} + (\beta_s^{n-1} (\alpha_s - \beta_s) + \tilde{\beta}_s) v_{max}^{(n)} \right) > \tilde{e} v_{max}^{(n)} > 0. \quad (\text{S56})$$

Hence, if

$$\left((\tilde{\alpha}_s^{n-1} - \alpha_s^{n-1}) r_{bas} + \beta_s^{n-1} v_{max}^{(n)} \right) < 0 \text{ for some } \tilde{x}, \tilde{y} \quad (\text{S57})$$

holds, then the partial with respect to \tilde{x} could change the sign for c large enough, more concretely,

$$c > 1 - \frac{\alpha_s^{n-1} \left(\bar{\alpha}_s^{n-1} (\alpha_s - \bar{\alpha}_s) + (\beta_s^{n-1} (\alpha_s - \beta_s) + \bar{\beta}_s) \frac{\nu_{max}^{(n)}}{r_{bas}} \right)}{(\bar{\alpha}_s^{n-1} - \alpha_s^{n-1}) r_{bas} + \beta_s^{n-1} \frac{\nu_{max}^{(n)}}{r_{bas}}}. \quad (S58)$$

Let us also observe that the inverse logic is not possible in the absence of total cooperativity, that is, $c > 1$ is required. Obviously, (S57) will occur whenever

$$\frac{\nu_{max}^{(n)}}{r_{bas}} < \sup_{\tilde{x}, \tilde{y}} \frac{(\alpha_s^{n-1} - \bar{\alpha}_s^{n-1})}{\beta_s^{n-1}} = \frac{1 - \tilde{r}^{n-1}}{(\tilde{e}\tilde{r})^{n-1}}$$

where the supremum can be easily calculated thanks to

$$\begin{aligned} \frac{(\alpha_s^{n-1} - \bar{\alpha}_s^{n-1})}{\beta_s^{n-1}} &\leq \frac{(\alpha_s^{n-1} - (\tilde{r}\alpha_s)^{n-1})}{(\tilde{e}\tilde{r}\alpha_s)^{n-1}} \\ &= \frac{1 - \tilde{r}^{n-1}}{(\tilde{e}\tilde{r})^{n-1}} = \lim_{\tilde{x} \rightarrow 0, \tilde{y} \rightarrow \infty} \frac{(\alpha_s^{n-1} - \bar{\alpha}_s^{n-1})}{\beta_s^{n-1}}. \end{aligned} \quad (S59)$$

Thus, we conclude always that

$$\frac{\nu_{max}^{(n)}}{r_{bas}} < \frac{1 - \tilde{r}^{n-1}}{(\tilde{e}\tilde{r})^{n-1}}$$

the set

$$H_{s,a} = \left\{ (\tilde{x}, \tilde{y}) \in (\mathbb{R}_0^+)^2 \mid \left((\bar{\alpha}_s^{n-1} - \alpha_s^{n-1}) r_{bas} + \beta_s^{n-1} \nu_{max}^{(n)} \right) |_{\tilde{x}, \tilde{y}} < 0 \right\}$$

is non empty and, for any

$$c > \tilde{c}_{th}^a = \inf_{H_{s,a}} \left\{ 1 - \frac{\alpha_s^{n-1} \left(\bar{\alpha}_s^{n-1} (\alpha_s - \bar{\alpha}_s) + (\beta_s^{n-1} (\alpha_s - \beta_s) + \bar{\beta}_s) \frac{\nu_{max}^{(n)}}{r_{bas}} \right)}{(\bar{\alpha}_s^{n-1} - \alpha_s^{n-1}) r_{bas} + \beta_s^{n-1} \frac{\nu_{max}^{(n)}}{r_{bas}}} \right\}$$

there exist points (\tilde{x}, \tilde{y}) where the sign on the right-hand side of (S54) is negative. Obviously, the value of c will determine the final set of values where it occurs by condition (S58).

- Repressors inverse logic for the Stimulated BEWARE operator
The sign of the partial derivative of the BEWARE operator with respect to the repressor variable is

$$\begin{aligned} &\text{sign} \left\{ \frac{\partial \text{BEWARE}_s([A], [R], [RNAP]; \{A, R\}_c)}{\partial [R]} \right\} \\ &= \text{sign} \left\{ \frac{r_{bas}}{1 + \frac{K_{RP}}{[RNAP]}} \frac{\partial \text{Basal}(\tilde{x}, \tilde{y})}{\partial \tilde{y}} + \frac{\nu_{max}^{(n)}}{1 + \frac{K_{RP}}{[RNAP]}} \frac{\partial \text{Promoter}(\tilde{x}, \tilde{y})}{\partial \tilde{y}} \right\} \\ &= \text{sign} \left\{ (c-1) \left((\bar{\alpha}_s^{n-1} \tilde{r} - \alpha_s^{n-1}) r_{bas} + \tilde{e}\tilde{r} (\beta_s^{n-1} - \bar{\beta}_s^{n-1}) \nu_{max}^{(n)} \right) \right. \\ &\quad \left. + \alpha_s^{n-1} \left(\bar{\alpha}_s^{n-1} (\tilde{r}\alpha_s - \bar{\alpha}_s) r_{bas} + (\tilde{e}\tilde{r} (\beta_s^{n-1} - \bar{\beta}_s^{n-1}) - (\beta_s^n - \bar{\beta}_s^n)) \nu_{max}^{(n)} \right) \right\}. \end{aligned} \quad (S60)$$

Here,

$$\alpha_s^{n-1} \left(\bar{\alpha}_s^{n-1} (\tilde{r}\alpha_s - \bar{\alpha}_s) r_{bas} + (\tilde{e}\tilde{r} (\beta_s^{n-1} - \bar{\beta}_s^{n-1}) - (\beta_s^n - \bar{\beta}_s^n)) \nu_{max}^{(n)} \right) < 0, \quad (S61)$$

since $\tilde{r}\alpha_s - \bar{\alpha}_s < 0$ by definition and

$$\tilde{e}\tilde{r}(\beta_s^{n-1} - \bar{\beta}_s^{n-1}) - (\beta_s^n - \bar{\beta}_s^n) = (\beta_s^n - \bar{\beta}_s^n) \left(\frac{H_{n-1}(\tilde{e}\tilde{r}\beta_s, \tilde{e}\tilde{r}\bar{\beta}_s)}{H_n(\beta_s, \bar{\beta}_s)} - 1 \right) < 0 \quad (\text{S62})$$

by (S49) and Lemma S3 ii). By this reason, if

$$(\bar{\alpha}_s^{n-1}\tilde{r} - \alpha_s^{n-1})r_{bas} + \tilde{e}\tilde{r}(\beta_s^{n-1} - \bar{\beta}_s^{n-1})\nu_{max}^{(n)} > 0, \quad (\text{S63})$$

then, for large enough c , more concretely when

$$c > 1 - \frac{\alpha_s^{n-1} \left(\bar{\alpha}_s^{n-1}(\tilde{r}\alpha_s - \bar{\alpha}_s) + (\tilde{e}\tilde{r}(\beta_s^{n-1} - \bar{\beta}_s^{n-1}) - (\beta_s^n - \bar{\beta}_s^n)) \frac{\nu_{max}^{(n)}}{r_{bas}} \right)}{(\bar{\alpha}_s^{n-1}\tilde{r} - \alpha_s^{n-1}) + \tilde{e}\tilde{r}(\beta_s^{n-1} - \bar{\beta}_s^{n-1}) \frac{\nu_{max}^{(n)}}{r_{bas}}} \quad (\text{S64})$$

the sign computed in (S60) can be positive. Again, this can be no longer true in the absence of cooperativity, that is, when $c = 1$. Now, (S63) can occur if and only if

$$\frac{r_{bas}}{\tilde{e}\tilde{r}\nu_{max}^{(n)}} < \sup \frac{\beta_s^{n-1} - \bar{\beta}_s^{n-1}}{\alpha_s^{n-1} - \tilde{r}\bar{\alpha}_s^{n-1}} = \frac{1}{1 - \tilde{r}} \quad (\text{S65})$$

where analogously, as was done before, the supremum can be easily calculated from

$$\frac{\beta_s^{n-1} - \bar{\beta}_s^{n-1}}{\alpha_s^{n-1} - \tilde{r}\bar{\alpha}_s^{n-1}} \leq \frac{\bar{\alpha}_s^{n-1}}{\bar{\alpha}_s^{n-1} - \tilde{r}\bar{\alpha}_s^{n-1}} = \frac{1}{1 - \tilde{r}} = \lim_{\tilde{x} \rightarrow \infty, \tilde{y} \rightarrow 0} \frac{\beta_s^{n-1} - \bar{\beta}_s^{n-1}}{\bar{\alpha}_s^{n-1}\tilde{r} - \alpha_s^{n-1}}.$$

Then, arguing as before, we can conclude that (S60) can be positive in the points determined by a set $H_{s,r}$ defined in terms of the condition defining by (S63) and for values c bigger than \tilde{c}_{th}^r determined by the lower term of (S64).

S4.2. Direct Logic in the Presence of Partial Cooperativity

Now, we prove that, in the presence of partial cooperativity between activators and/or repressors, the inverse logic can not occur as stated in Lemma (S2) ii). In the rest of this proof, we will denote $\tilde{x} = \frac{c_A[A]}{K_A}$ and $\tilde{y} = \frac{c_R[R]}{K_R}$.

- Activator direct logic for the partial cooperative recruitment BEWARE operator
Using the increasing character of the recruitment BEWARE operator with respect to the regulation factor operator, we have

$$\begin{aligned} \text{sign} \left\{ \frac{\partial \text{BEWARE}_r([A], [R], [RNAP]; \{A\}_{c_A}, \{R\}_{c_R})}{\partial [A]} \right\} &= \text{sign} \left\{ \frac{\partial F_{reg}(\tilde{x}, \tilde{y})}{\partial \tilde{x}} \right\} \\ &= \text{sign} \left\{ (c_A - 1)(c_R - 1)^2 (a\gamma_r^{n-1} - \bar{\gamma}_r^{n-1}) + (c_R - 1)^2 \gamma_r^{n-1} \bar{\gamma}_r^{n-1} (a\bar{\gamma}_r - \gamma_r) \right. \\ &\quad + \bar{\alpha}_r^{n-1} \alpha_r^{n-1} (a\alpha_r - \bar{\alpha}_r) + (c_A - 1) \left(\frac{a}{\bar{\alpha}_r} \bar{\alpha}_r^n \beta_r^n - \frac{1}{\alpha_r} \alpha_r^n \bar{\beta}_r^n \right) \\ &\quad + (c_R - 1) \left(\bar{\alpha}_r^n \bar{\gamma}_r^n \left(\frac{a}{\bar{\alpha}_r} - \frac{1}{\bar{\gamma}_r} \right) + \gamma_r^n \alpha_r^n \left(\frac{a}{\gamma_r} - \frac{1}{\alpha_r} \right) \right) \\ &\quad \left. + (c_A - 1)(c_R - 1) (a\bar{\alpha}_r^{n-1} - \alpha_r^{n-1} + a\gamma_r^{n-1} \beta_r^n - \bar{\gamma}_r^{n-1} \bar{\beta}_r^n) \right\} \quad (\text{S66}) \end{aligned}$$

where $\bar{\alpha}_r = \bar{\alpha}_r(\tilde{x}, \tilde{y}) = 1 + a\tilde{x} + r\tilde{y}$, $\alpha_r = \alpha_r(\tilde{x}, \tilde{y}) = 1 + \tilde{x} + \tilde{y}$, $\bar{\beta}_r = \bar{\beta}_r(\tilde{y}) = 1 + r\tilde{y}$, $\beta_r = \beta_r(\tilde{y}) = 1 + \tilde{y}$, $\bar{\gamma}_r = \bar{\gamma}_r(\tilde{x}) = 1 + \tilde{x}$, $\gamma_r = \gamma_r(\tilde{x}) = 1 + a\tilde{x}$. From these definitions,

we can easily check that almost all the terms inside the sign function are positive by using the estimates:

$$\begin{aligned} \bar{\alpha}_r &\geq \gamma_r \geq \bar{\gamma}_r, & a\bar{\gamma}_r &\geq \gamma_r, & \frac{a}{\bar{\alpha}_r} &\geq \frac{1}{\alpha_r}, & \frac{a}{\gamma_r} &\geq \frac{1}{\alpha_r}, \\ \bar{\alpha}_r\beta_r &\geq \alpha_r\bar{\beta}_r, & \alpha_r\gamma_r &\geq \bar{\alpha}_r\bar{\gamma}_r, & \frac{a}{\gamma_r} &\geq \frac{1}{\bar{\gamma}_r}, & \alpha_r &\geq \beta_r \geq \bar{\beta}_r. \end{aligned} \quad (\text{S67})$$

The positivity of the last term can be checked by observing that the expression $(a\bar{\alpha}_r^{n-1} - \alpha_r^{n-1} + a\gamma_r^{n-1}\beta_r^n - \bar{\gamma}_r^{n-1}\bar{\beta}_r^n)$ is increasing with respect to the variable a . Then, this will be always positive if it is for $a = 1$. In this sense, we estimate

$$\begin{aligned} (1 + \tilde{x} + r\tilde{y})^{n-1} - (1 + \tilde{x} + \tilde{y})^{n-1} + (1 + \tilde{x})^{n-1}(1 + \tilde{y})^n - (1 + \tilde{x})^{n-1}(1 + r\tilde{y})^n \\ \geq \left(1 + r\frac{\tilde{y}}{1 + \tilde{x}}\right)^{n-1} - \left(1 + \frac{\tilde{y}}{1 + \tilde{x}}\right)^{n-1} + (1 + \tilde{y})^{n-1} - (1 + r\tilde{y})^{n-1} \end{aligned}$$

where the lower term is positive since, using (S49), we obtain

$$\begin{aligned} (1 + \tilde{y})^{n-1} - \left(1 + \frac{\tilde{y}}{1 + \tilde{x}}\right)^{n-1} &\geq (1 + r\tilde{y})^{n-1} - \left(1 + r\frac{\tilde{y}}{1 + \tilde{x}}\right)^{n-1} \\ \iff H_{n-1}\left(1 + \tilde{y}, 1 + \frac{\tilde{y}}{1 + \tilde{x}}\right) &\geq rH_{n-1}\left(1 + r\tilde{y}, 1 + r\frac{\tilde{y}}{1 + \tilde{x}}\right) \end{aligned} \quad (\text{S68})$$

which holds because of the increasing character of the operator H_{n-1} .

- Repressor direct logic for the partial cooperative recruitment BEWARE operator

The fact that the partial derivative of BEWARE_r with respect to the repressor variable is negative when partial cooperativity between activator and repressor can be verified in an analogous manner by the symmetric roles of activators and repressors in the recruitment operators.

- Activator direct logic for the partial cooperative stimulated BEWARE operator
Since $\text{Basal}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\})$ has the same expression as the Regulation Factor (S13) with $a = 1$, the sign of the derivatives can be estimated following exactly the deduction for the recruitment operator. Hence, we need to check the sign only for the partial derivatives of the $\text{Promoter}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\})$ function

$$\frac{(c_R - 1)(\delta_s^n - \bar{\delta}_s^n) + \beta_s^n - \bar{\beta}_s^n}{(c_A - 1)(c_R - 1) + \alpha_s^n + (c_R - 1)\lambda_s^n + (c_A - 1)\gamma_s^n}$$

in order to conclude the proof of Lemma S2. In the previous definition, we have used $\alpha_s = \alpha_s(\tilde{x}, \tilde{y}) = 1 + \tilde{x} + \tilde{y}$, $\bar{\alpha}_s = \bar{\alpha}_s(\tilde{x}, \tilde{y}) = 1 + \tilde{x} + r\tilde{y}$, $\beta_s = \beta_s(\tilde{x}, \tilde{y}) = \tilde{e} + \tilde{x} + \tilde{e}\tilde{r}\tilde{y}$, $\bar{\beta}_s = \bar{\beta}_s(\tilde{y}) = \tilde{e} + \tilde{e}\tilde{r}\tilde{y}$, $\gamma_s = \gamma_s(\tilde{y}) = 1 + \tilde{y}$, $\bar{\gamma}_s = \bar{\gamma}_s(\tilde{y}) = 1 + r\tilde{y}$, $\delta_s = \delta_s(\tilde{x}) = \tilde{e} + \tilde{x}$, $\bar{\delta}_s = \bar{\delta}_s = \tilde{e}$, $\lambda_s = \lambda_s(\tilde{x}) = 1 + \tilde{x}$ where, again, we are assuming that each function is evaluated on $\tilde{x} = \frac{c_A[A]}{K_A}$ and $\tilde{y} = \frac{c_R[R]}{K_R}$. The sign of the partial derivative with respect the activator variable will come from the sign of

$$\begin{aligned} \text{sign}\left\{\frac{\partial \text{BEWARE}_s([A], [R]), [RNAP]; \{\{A\}_{c_A}, \{R\}_{c_R}\})}{\partial [A]}\right\} \\ = \text{sign}\left\{-\left(\alpha_s^{n-1} + (c_R - 1)\lambda_s^{n-1}\right)\left((c_R - 1)(\delta_s^n - \bar{\delta}_s^n) + \beta_s^n - \bar{\beta}_s^n\right) \right. \\ \left. + \left((c_R - 1)\delta_s^{n-1} + \beta_s^{n-1}\right)\left((c_A - 1)(c_R - 1) + \alpha_s^n + (c_R - 1)\lambda_s^n + (c_A - 1)\gamma_s^n\right)\right\} \end{aligned}$$

which, rearranging the terms, translates to check the sign of

$$\begin{aligned} & \text{sign} \left\{ (c_R - 1)^2 \left((c_A - 1) \delta_s^{n-1} + \lambda_s^{n-1} (\lambda_s \delta_s^{n-1} - \delta_s^n + \bar{\delta}_s^n) \right) \right. \\ & + \alpha_s^{n-1} (\beta_s^{n-1} \alpha_s - \beta_s^n - \bar{\beta}_s^n) \\ & + (c_R - 1)(c_A - 1) \left(\delta_s^{n-1} \gamma_s^n + \beta_s^{n-1} \right) + (c_A - 1) \gamma_s^n \beta_s^{n-1} \\ & \left. + (c_R - 1) \left(\delta_s^{n-1} \alpha_s^n + \lambda_s^n \beta_s^{n-1} - \alpha_s^{n-1} (\delta_s^n - \bar{\delta}_s^n) - \lambda_s^{n-1} (\beta_s^n - \bar{\beta}_s^n) \right) \right\}. \end{aligned}$$

The first terms are trivially positive since $\lambda_s > \delta_s$ and $\alpha_s > \beta_s$. In addition, it is positive that the last term can be rewritten, up to the multiplicative constant $(c_R - 1)$, as

$$\lambda_s^{n-1} \beta_s^{n-1} (\lambda_s - \beta_s) + \alpha_s^{n-1} \delta_s^{n-1} (\alpha_s - \delta_s) + \lambda_s^{n-1} \bar{\beta}_s^{n-1} + \alpha_s^{n-1} \bar{\delta}_s^n \geq 0$$

thanks to $|\lambda_s - \beta_s| \leq (\alpha_s - \delta_s)$ and $\alpha_s \delta_s \geq \lambda_s \beta_s$.

- Repressor direct logic for the partial cooperative stimulated BEWARE operator
On the other hand,

$$\begin{aligned} & \text{sign} \left\{ \frac{\partial \text{BEWARE}_s([A], [R]), [RNAP]; \{\{A\}_{c_A}, \{R\}_{c_R}\})}{\partial [R]} \right\} \\ & = \text{sign} \left\{ \left(\tilde{e} \tilde{r} (\beta_s^{n-1} - \bar{\beta}_s^{n-1}) \right) \left((c_A - 1)(c_R - 1) + \alpha_s^n + (c_R - 1) \lambda_s^n + (c_A - 1) \gamma_s^n \right) \right. \\ & \left. - \left(\alpha_s^{n-1} + (c_A - 1) \gamma_s^{n-1} \right) \left((c_R - 1) (\delta_s^n - \bar{\delta}_s^n) + \beta_s^n - \bar{\beta}_s^n \right) \right\} \end{aligned}$$

and, rearranging the terms, the same sign can be obtained from

$$\begin{aligned} & \text{sign} \left\{ (c_A - 1)(c_R - 1) \left(\tilde{e} \tilde{r} (\beta_s^{n-1} - \bar{\beta}_s^{n-1}) - \gamma_s^{n-1} (\delta_s^n - \bar{\delta}_s^n) \right) \right. \\ & + (c_R - 1) \left((\tilde{e} \tilde{r} \lambda_s) (\lambda_s \beta_s)^{n-1} \left(1 - \frac{\bar{\beta}_s^{n-1}}{\beta_s^{n-1}} \right) + \delta_s (\delta_s \alpha_s)^{n-1} \left(\frac{\bar{\delta}_s^n}{\delta_s^n} - 1 \right) \right) \\ & + (c_A - 1) \left((\gamma_s \beta_s)^{n-1} (\tilde{e} \tilde{r} \gamma_s - \beta_s) + (\gamma_s \bar{\beta}_s)^{n-1} (\bar{\beta}_s - \tilde{e} \tilde{r} \gamma_s) \right) \\ & \left. + (\alpha_s \beta_s)^{n-1} (\tilde{e} \tilde{r} \alpha_s - \beta_s) + (\alpha_s \bar{\beta}_s)^{n-1} (\bar{\beta}_s - \tilde{e} \tilde{r} \alpha_s) \right\}. \end{aligned} \quad (\text{S69})$$

The first term in (S69)

$$\begin{aligned} & \tilde{e} \tilde{r} (\beta_s^{n-1} - \bar{\beta}_s^{n-1}) - \gamma_s^{n-1} (\delta_s^n - \bar{\delta}_s^n) \\ & = (\delta_s - \bar{\delta}_s) (\tilde{e} \tilde{r} H_{n-1}(\beta_s, \bar{\beta}_s) - \gamma_s^{n-1} H_n(\delta_s, \bar{\delta}_s)) \\ & = (\delta_s - \bar{\delta}_s) (\tilde{e} \tilde{r} H_{n-1}(\beta_s, \bar{\beta}_s) - H_n(\gamma_s \delta_s, \gamma_s \bar{\delta}_s)) \\ & = (\delta_s - \bar{\delta}_s) H_n(\gamma_s \delta_s, \gamma_s \bar{\delta}_s) \left(\frac{\tilde{e} \tilde{r} H_{n-1}(\beta_s, \bar{\beta}_s)}{H_n(\gamma_s \delta_s, \gamma_s \bar{\delta}_s)} - 1 \right) \\ & \leq (\delta_s - \bar{\delta}_s) H_n(\gamma_s \delta_s, \gamma_s \bar{\delta}_s) \left(\frac{\tilde{e} \tilde{r} H_{n-1}(\gamma_s \delta_s, \gamma_s \bar{\delta}_s)}{H_n(\gamma_s \delta_s, \gamma_s \bar{\delta}_s)} - 1 \right) \leq 0 \end{aligned}$$

is negative because $\beta_s \leq \gamma_s \delta_s$, $\bar{\beta}_s \leq \gamma_s \bar{\delta}_s$ and Lemma S49 iii) is used being $\tilde{e} \tilde{r} \leq \gamma_s \delta_s$. The rest of the terms in (S69) can be proven to be negative following the next estimates

$$\begin{aligned} & (\tilde{e} \tilde{r} \lambda_s) (\lambda_s \beta_s)^{n-1} \left(1 - \frac{\bar{\beta}_s^{n-1}}{\beta_s^{n-1}} \right) + \delta_s (\delta_s \alpha_s)^{n-1} \left(\frac{\bar{\delta}_s^n}{\delta_s^n} - 1 \right) \leq \delta_s (\delta_s \alpha_s)^{n-1} \left(\frac{\bar{\delta}_s^n}{\delta_s^n} - \frac{\bar{\beta}_s^{n-1}}{\beta_s^{n-1}} \right) \\ & (\gamma_s \beta_s)^{n-1} (\tilde{e} \tilde{r} \gamma_s - \beta_s) + (\gamma_s \bar{\beta}_s)^{n-1} (\bar{\beta}_s - \tilde{e} \tilde{r} \gamma_s) \leq (\gamma_s \bar{\beta}_s)^{n-1} (\bar{\beta}_s - \beta_s) \\ & (\alpha_s \beta_s)^{n-1} (\tilde{e} \tilde{r} \alpha_s - \beta_s) + (\alpha_s \bar{\beta}_s)^{n-1} (\bar{\beta}_s - \tilde{e} \tilde{r} \alpha_s) \leq (\alpha_s \bar{\beta}_s)^{n-1} (\bar{\beta}_s - \beta_s) \end{aligned}$$

and thanks to

$$\begin{aligned} \alpha_s > \gamma_s \geq \bar{\gamma}_s, \quad \lambda_s \beta_s \leq \alpha_s \delta_s, \quad \tilde{e}\tilde{r}\lambda_s \leq \delta_s, \quad 1 \geq \frac{\bar{\beta}_s}{\beta_s} \geq \frac{\bar{\delta}_s}{\delta_s}, \\ \alpha_s > \delta_s \geq \bar{\delta}_s, \quad \bar{\beta}_s \leq \beta_s, \quad \tilde{e}\tilde{r}\gamma_s \leq \beta_s, \quad \tilde{e}\tilde{r}\alpha_s \leq \beta_s. \end{aligned} \quad (\text{S70})$$

□

S5. Existence of Threshold

This Section is devoted to proving the existence of activation/repression thresholds implicitly deduced from the BEWARE operators.

Proposition S2. *In the global activator/repressor framework of each BEWARE operator, a unique, positive and increasing function $[R] = f_{m,l}([A]; n)$ is determined fulfilling Equation (5) in the main manuscript. This function determines the threshold between two regions in the plane $([A], [R])$. Each region contains concentrations providing transcriptional levels either over the basal level, if $[R] < f_{m,l}([A]; n)$, or under the basal level, when $[R] > f_{m,l}([A]; n)$.*

These threshold functions depend on all the biochemical factors considered in the derivation of the BEWARE operator: the Recruitment ($m = r$) (S12) or Stimulated ($m = s$) (S15) (with $r_{bas} > 0$) approaches, the binding cooperativity mechanisms between the TFs (cooperative, total cooperative $l = t$ or partial cooperative $l = p$) or the number of enhancers (n). The particular case of the dependence with respect to the affinities' coefficients K_A - K_R is given by

$$f_{m,l}([A]; n) := K_R \tilde{f}_{m,l} \left(\frac{[A]}{K_A}; n \right) \quad (\text{S71})$$

where $\tilde{f}_{m,l}$ is independent of both K_A and K_R .

Remark S6. *A very easy example can be shown in the case of the BEWARE operators with null/total cooperativity (S8). Because of the increasing character of the recruitment operator (S12), with respect to the regulation factor F_{reg} , Equation (5) translates directly to*

$$F_{reg}([A], f_{r,t}([A]; n); \mathcal{C}) = F_{reg}((0, 0); \mathcal{C}) = 1. \quad (\text{S72})$$

When we substitute the definition of the regulation factor (S39) in this expression, we directly obtain that the threshold corresponds to the linear expression

$$f_{r,t}([A]; n) = \frac{K_R}{K_A} \frac{a-1}{1-r} [A].$$

Let us remark that, although in expression (S39) the cooperativity constant c and the number of enhancers n are present, they are not in this threshold expression. This implies that the thresholds for this model are the independent of the intensity of the total binding cooperativity between the species or the number of enhancers.

Remark S7. *A similar argument can be performed for the stimulated BEWARE operator when $n = 1$. Obviously, this is independent of any kind of binding cooperativity because it can not occur when only one binding site is available. In this case, direct computations give rise to*

$$f_{s,t}([A]; 1) = \frac{K_R}{K_A} \frac{v_{max}^{(1)}}{r_{bas}} \frac{1}{1-\tilde{r}} [A].$$

As we will mention subsequently, the thresholds under the stimulated approach with total cooperativity are not in general independent of the number of enhancers, which will represent a remarkable difference between the stimulated and recruitment approach.

Remark S8. Proposition S2 is no longer true for Stimulated operators when r_{bas} is null as can be trivially deduced from expressions (S41) and (S45). In this case, the threshold coincides with the axis $[A] = 0$, and any pair of concentrations $([A], [R])$ with $[A] > 0$ leads to activation levels. Nevertheless, we have to remark that this threshold does not depend on cooperativity relations, number of enhancers or affinities.

Sketch of the Proof of Proposition S2

Here, we have adopted the notation $x = \frac{[A]}{K_A}$, $y = \frac{[R]}{K_R}$ for convenience. Let us mention that the threshold for the recruitment BEWARE operator with n enhancers and null/total cooperativity was already calculated in Remark S6 solving explicitly the equation

$$G_{r,t}(x, y; n) = (1 + acx + rcy)^n - (1 + cx + cy)^n = 0 \quad (S73)$$

which is equivalent to (S72). In current notation, the solution to this equation is given by:

$$y = \tilde{f}_{r,t}(x; n) = \frac{a-1}{1-r}x, \quad (S74)$$

where $\tilde{f}_{r,t}(x; n)$ is the function stated in (S71). Now, undoing our original change of variable $x = \frac{[A]}{K_A}$, $y = \frac{[R]}{K_R}$, we recover the expression deduced in Remark S6.

Although in this case the definition of these thresholds can be done explicitly, we would like to remark that the general existence result provided by the implicit function theorem provides very useful information for subsequent analysis. The argument we adopt follows the same scheme for all the BEWARE functionals considered, so we now introduce the outlines of the general proof and in sub-S5.1 we check the validity of each particular model dependent requirements.

When we substitute the concrete expressions of the BEWARE operators (S12)–(S15) with their corresponding expressions for regulation factors, basal and promoter functions (see (S39)–(S45)) into Equation (5), this is equivalent to an equation $G_{m,l}\left(\frac{[A]}{K_A}, \frac{f([A])}{K_R}; n\right) = 0$. In the case of the recruitment BEWARE operators, we obtain

$$\begin{aligned} G_{r,t}(x, y; n) &= \bar{\alpha}_r(cx, cy)^n - \alpha_r(cx, cy)^n, \\ G_{r,p}(x, y; n) &= \bar{\alpha}_r(c_A x, c_R y)^n - \alpha_r(c_A x, c_R y)^n + (c_A - 1)(\bar{\beta}_r(c_R y)^n - \beta_r(c_R y)^n) \\ &\quad + (c_R - 1)(\gamma_r(c_A x)^n - \bar{\gamma}_r(c_A x)^n), \end{aligned} \quad (S75)$$

being $\bar{\alpha}_r(X, Y) = (1 + aX + rY)$, $\alpha_r(X, Y) = (1 + X + Y)$, $\bar{\beta}_r(Y) = 1 + rY$, $\beta_r(Y) = 1 + Y$, $\bar{\gamma}_r(X) = 1 + X$ and $\gamma_r(X) = 1 + aX$. In the stimulated cases, we obtain in the same way

$$\begin{aligned} G_{s,t}(x, y; n) &= r_{bas}(\bar{\alpha}_s^n(cx, cy) - \alpha_s^n(cx, cy)) + v_{max}^{(n)}(\beta_s^n(cx, cy) - \bar{\beta}_s^n(cy)), \\ G_{s,p}(x, y; n) &= r_{bas}(\bar{\alpha}_s^n(c_A x, c_R y) - \alpha_s^n(c_A x, c_R y) + (c_A - 1)(\bar{\gamma}_s^n(c_R y) - \gamma_s^n(c_R y))) \\ &\quad + v_{max}^{(n)}(\beta_s^n(c_A x, c_R y) - \bar{\beta}_s^n(c_R y) + (c_R - 1)(\delta_s^n(c_A x) - \bar{\delta}_s^n)) \end{aligned} \quad (S76)$$

where now $\bar{\alpha}_s(X, Y) = 1 + X + \tilde{r}Y$, $\alpha_s(X, Y) = 1 + X + Y$, $\bar{\beta}_s(Y) = \tilde{e} + \tilde{e}\tilde{r}Y$, $\beta_s(X, Y) = \tilde{e} + X + \tilde{e}\tilde{r}Y$, $\bar{\gamma}_s(Y) = 1 + \tilde{r}Y$, $\gamma_s(Y) = 1 + Y$, $\bar{\delta}_s = \tilde{e}$ and $\delta_s(X) = \tilde{e} + X$. As it has been done in (S73)–(S74), G functions and the corresponding thresholds f will be denoted with subindices: s, r, t, p , corresponding to stimulated, recruitment, total/null cooperativity and partial cooperativity, respectively. Some other dependences can be included whenever necessary by using parameters, as, for instance, $G_{m,l}(\cdot, \cdot; n)$ determining that G is a bivariate polynomial function of order n . Subsequently, subindexes as well as the parameter will be skipped in all those cases where they are not relevant. Let us also notice that all the functions G have been defined such that

$$G\left(\frac{[A]}{K_A}, \frac{[R]}{K_R}\right) > 0 \iff \text{BEWARE}([A], [R]) > \text{basal level}. \quad (S77)$$

In order to prove the existence of an implicit function determined by the equation $G(x, y) = 0$ for each of these problems, we propose to check the next items:

a) for any $x > 0$, the equation

$$G(x, \cdot) = 0 \quad (\text{S78})$$

function has at least one root because $G(x, 0) > 0$ and $\lim_{y \rightarrow \infty} G(x, y) = -\infty$,

b) for any (x, y) root of the equation (S78), then $\frac{\partial G}{\partial y}(x, y) < 0$,

c) and, for any (x, y) , the root of the equation (S78), then $\frac{\partial G}{\partial x}(x, y) > 0$.

For instance, items a), b) and c) can be easily checked for $G_{r,t}$ defined in (S73) thanks to $r < 1 < a$. These ingredients allow us to conclude the proof easily. Assertion b) implies the uniqueness of the roots stated in a) because $G(x, \cdot)$ is strictly decreasing at any of them. Since the partial derivative with respect to y is non-zero, the implicit function theorem assures that, given a point (x_1, y_1) such that $G(x_1, y_1) = 0$, then in some small enough neighbourhood of (x_1, y_1) , there exists a parametrisation $(x, \tilde{f}(x))$ such that $G(x, \tilde{f}(x)) = 0$. This really justifies that the function $\tilde{f}(x)$ is globally defined and unique. Finally, this function is also monotone increasing because of b) and c), since

$$\tilde{f}'(x) = -\frac{\frac{\partial G}{\partial x}}{\frac{\partial G}{\partial y}} > 0 \quad (\text{S79})$$

Let us mention that the functions \tilde{f} are independent of the affinity constants K_A and K_R because of the initial change of variables $x = \frac{[A]}{K_A}$, $y = \frac{[R]}{K_R}$ and Remark S1. Let us see that these functions define the thresholds. Coming back to our original notation, the function f stated in Proposition S2 takes the value expression given by (S71). These thresholds will depend as much as from the cooperative relations between TFs: not cooperative/total cooperative (t) or partial cooperative (p) as from the recruitment (r) or stimulated (s) approaches reason why we will add subindexes $t/p, r/s$ to f function denoting any threshold, as it was done in definition (S74). It is also true that the thresholds will depend on the number of enhancers n , which will be introduced as a parameter dependence $f_{m,l}([A]; n)$. \square

S5.1. Existence of Thresholds for Bifunctional Beware Operators in the Activator/Repressor Framework

The rest of the Section is devoted to declaring the functions G determining the activation/repression thresholds and checking the hypothesis a) – c) they have to verify in order to conclude Proposition S2 for all the considered BEWARE models.

Proof of Proposition S2

- Stimulated BEWARE operator with null/total cooperativity ($r_{bas} > 0$)
From definitions (S15) and the corresponding basal level, see Table S1, we obtain that Equation (5) in this case study translates into

$$r_{bas}(\text{Basal}([A], [R]); C) - 1 + v_{max}^{(n)} \text{Promoter}([A], [R]); C = 0. \quad (\text{S80})$$

Replacing definition Basal and Promoter functions according to expressions (S40) and (S41), equation (S80) leads to

$$\begin{aligned} G_{s,t}(x, y; n) \\ = r_{bas}(\bar{\alpha}_s^n(cx, cy) - \alpha_s^n(cx, cy)) + v_{max}^{(n)}(\beta_s^n(cx, cy) - \bar{\beta}_s^n(cy)) = 0, \end{aligned} \quad (\text{S81})$$

where $x = \frac{[A]}{K_A}$, $y = \frac{[R]}{K_R}$, $\bar{\alpha}_s(X, Y) = 1 + X + \bar{r}Y$, $\alpha_s(X, Y) = 1 + X + Y$, $\bar{\beta}_s(Y) = \bar{e} + \bar{e}\bar{r}Y$, $\beta_s(X, Y) = \bar{e} + X + \bar{e}\bar{r}Y$. Let us assume in the subsequent that $n \geq 2$ since Proposition S2 is obviously true for $n = 1$. For simplicity, we will note $\alpha_s = \alpha_s(cx, cy)$, $\bar{\alpha}_s = \bar{\alpha}_s(cx, cy)$, $\beta_s = \beta_s(cx, cy)$ and $\bar{\beta}_s = \bar{\beta}_s(cy)$. Using estimates (S55), it is easy to

prove a), that is, $G_{s,t}(x, 0; n) > 0$ and $\lim_{y \rightarrow +\infty} G_{s,t}(x, y; n) = -\infty$, so Equation (S81) has at least one real root.

A similar procedure can be used in order to prove b). In this case,

$$\begin{aligned} & \text{sign} \left\{ \frac{\partial G_{s,t}}{\partial y} \right\} \\ &= \text{sign} \left\{ \left(r_{bas} \left(\tilde{r} \tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) + v_{max}^{(n)} \tilde{e} \tilde{r} \left(\beta_s^{n-1} - \tilde{\beta}_s^{n-1} \right) \right) \right\}. \end{aligned} \quad (\text{S82})$$

Obviously, b) holds when $n = 1$ because $\tilde{r} < 1$. When $n > 1$, let us observe that we know that under certain circumstances the right-hand side on (S82) can be positive by (S65). Nevertheless, we can prove that this does not occur at the solutions of (S81). From (S65), we obtain by simple algebra

$$\begin{aligned} & y \left(r_{bas} \left(\tilde{r} \tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) + v_{max}^{(n)} \tilde{e} \tilde{r} \left(\beta_s^{n-1} - \tilde{\beta}_s^{n-1} \right) \right) \\ &= -r_{bas} (1+x) \left(\tilde{r} \tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) - v_{max}^{(n)} \tilde{e} \left(\beta_s^{n-1} - \tilde{\beta}_s^{n-1} \right) - v_{max}^{(n)} x \beta_s^{n-1} \\ &< -(1+x) \left(r_{bas} \left(\tilde{r} \tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) + v_{max}^{(n)} \tilde{e} \tilde{r} \left(\beta_s^{n-1} - \tilde{\beta}_s^{n-1} \right) \right). \end{aligned} \quad (\text{S83})$$

On the other hand, we also can directly overestimate $\tilde{r} \tilde{\alpha}_s^{n-1}$ by taking $\tilde{r} = 1$ since $\tilde{r} < 1$ and $\tilde{\alpha}_s > 0$

$$\begin{aligned} & \left(r_{bas} \left(\tilde{r} \tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) + v_{max}^{(n)} \tilde{e} \tilde{r} \left(\beta_s^{n-1} - \tilde{\beta}_s^{n-1} \right) \right) \\ &\leq \left(r_{bas} \left(\tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) + v_{max}^{(n)} \tilde{e} \tilde{r} \left(\beta_s^{n-1} - \tilde{\beta}_s^{n-1} \right) \right). \end{aligned} \quad (\text{S84})$$

Since the upper bound in (S83) coincides with the upper bound in (S84) multiplied by a negative constant, we can deduce that the right-hand side on (S82) is negative for $y > 0$. That is, b) is verified.

In order to check c), we have that

$$\text{sign} \left\{ \frac{\partial G_{s,t}}{\partial x} \right\} = \text{sign} \left\{ \left(r_{bas} \left(\tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) + v_{max}^{(n)} \beta_s^{n-1} \right) \right\}. \quad (\text{S85})$$

Now, by using (S49) and (S81) as before, we obtain

$$\begin{aligned} & \left(v_{max}^{(n)} \beta_s^{n-1} + r_{bas} \left(\tilde{\alpha}_s^{n-1} - \alpha_s^{n-1} \right) \right) \\ &= v_{max}^{(n)} \left(\beta_s^{n-1} - \frac{H_{n-1}(\alpha_s, \tilde{\alpha}_s)}{H_n(\alpha_s, \tilde{\alpha}_s)} (\beta_s^n - \tilde{\beta}_s^n) \right) \\ &= v_{max}^{(n)} \beta_s^{n-1} \left(1 - \frac{H_{n-1}(\alpha_s, \tilde{\alpha}_s)}{H_n(\alpha_s, \tilde{\alpha}_s)} \beta_s \right) \\ &+ v_{max}^{(n)} \frac{H_{n-1}(\alpha_s, \tilde{\alpha}_s)}{H_n(\alpha_s, \tilde{\alpha}_s)} \tilde{\beta}_s^n, \end{aligned}$$

which is trivially positive, thanks to Lemma S3 iii) using $\beta_s < \alpha_s$ from (S55). Hence,

$\left. \frac{\partial G_{s,t}}{\partial x} \right|_{G_{s,t}=0} > 0$. Thus, we can assert the existence of a unique increasing function $\tilde{f}_{s,t}$ such that

$$G_{s,t}(x, \tilde{f}_{s,t}(x; n); n) = 0 \quad x \in (0, \infty) \quad (\text{S86})$$

for any $c > 0$.

- Stimulated BEWARE operators with partial cooperativity ($r_{bas} > 0$)
Now, in the case of BEWARE operators in the presence of partial cooperativity, the proofs are much easier because we can take advantage of the direct activator/repressor logic verified by these models as was stated in Lemma S2.

The expression defining the threshold condition (5) leads to:

$$\text{BEWARE}_s([A], [R], [RNAP]; \{A\}_{c_A}, \{R\}_{c_R}) - \frac{r_{bas}}{1 + \frac{K_{RP}}{[RNAP]}} = 0.$$

Then, multiplying this equation by $1 + \frac{K_{RP}}{[RNAP]}$ times, the denominator of the basal and promoter expressions, $\text{Den}_s([A], [R])$, and substituting the corresponding expressions of the basal and promoter functionals, (S43) and (S45), we obtain

$$\begin{aligned} 0 &= \text{Den}_s([A], [R]) \times \\ &\times \left(\left(1 + \frac{K_{RP}}{[RNAP]} \right) \text{BEWARE}_s([A], [R], [RNAP]; \{A\}_{c_A}, \{R\}_{c_R}) - r_{bas} \right) \\ &= r_{bas} (\bar{\alpha}_s^n(c_A x, c_R y) - \alpha_s^n(c_A x, c_R y) + (c_A - 1)(\bar{\gamma}_s^n(c_R y) - \gamma_s^n(c_R y))) \\ &+ \nu_{max}^{(n)} (\bar{\beta}_s^n(c_A x, c_R y) - \beta_s^n(c_R y) + (c_R - 1)(\bar{\delta}_s^n(c_A x) - \delta_s^n)) \\ &= G_{s,p}(x, y; n), \end{aligned} \quad (\text{S87})$$

where, in addition to the functions α_s , $\bar{\alpha}_s$, β_s and $\bar{\beta}_s$ appearing in (S81), we now also have $\bar{\gamma}_s(Y) = 1 + \bar{r}Y$, $\gamma_s(Y) = 1 + Y$, $\bar{\delta}_s = \bar{e}$ and $\delta_s(X) = \bar{e} + X$. Equation (S87) has at least one real root for any $x > 0$, since $G_{s,p}(x, 0; n) > 0$ and $\lim_{y \rightarrow +\infty} G_{s,p}(x, y; n) = -\infty$, that is, *a*) holds.

We can now check *b*) and *c*) very easily by observing that

$$\begin{aligned} \text{sign} \left\{ \frac{\partial G_{s,p}}{\partial y} \Big|_{G_{s,p}=0} \right\} &= \text{sign} \left\{ \frac{\partial \text{BEWARE}_s}{\partial [R]}([A], [R], [RNAP]; \{A\}_{c_A}, \{R\}_{c_R}) \right\} < 0, \\ \text{sign} \left\{ \frac{\partial G_{s,p}}{\partial x} \Big|_{G_{s,p}=0} \right\} &= \text{sign} \left\{ \frac{\partial \text{BEWARE}_s}{\partial [A]}([A], [R], [RNAP]; \{A\}_{c_A}, \{R\}_{c_R}) \right\} > 0, \end{aligned}$$

thanks to identities (S87) and Lemma S2 ii) proved in S4.2.

- Recruitment BEWARE operators with partial cooperativity
As it was introduced in Remark S6, the threshold equation, (5), translates directly to (S72) for Recruitment BEWARE operators.
Then, by multiplying this equation by the denominator of the regulation factor (S42), $\text{Den}_r([A], [R])$, we obtain the equivalent expression

$$\begin{aligned} 0 &= \text{Den}_r([A], [R]) \left(F_{reg}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\} - 1 \right) \\ &= \bar{\alpha}_r(c_A x, c_R y)^n - \alpha_r(c_A x, c_R y)^n + (c_A - 1)(\bar{\beta}_r(c_R y)^n - \beta_r(c_R y)^n) \\ &+ (c_R - 1)(\bar{\gamma}_r(c_A x)^n - \gamma_r(c_A x)^n) = G_{r,p}(x, y; n) \end{aligned} \quad (\text{S88})$$

with $\bar{\alpha}_r(X, Y) = (1 + aX + rY)$, $\alpha_r(X, Y) = (1 + X + Y)$, $\bar{\beta}_r(Y) = 1 + rY$, $\beta_r(Y) = 1 + Y$, $\bar{\gamma}_r(X) = 1 + X$ and $\gamma_r(X) = 1 + aX$. Note that Equation (S88) verifies *a*) since $G_{r,p}(x, 0; n) > 0$ and $\lim_{y \rightarrow +\infty} G_{r,p}(x, y; n) = -\infty$ for any $x > 0$ thanks to $a > 1$ and $r < 1$ are assumed.

In this case, the testing $b) - c)$ is again trivial by using identities (S88) and Lemma S2 ii) as indicated

$$\begin{aligned}\operatorname{sign}\left\{\frac{\partial G_{r,p}}{\partial y}\bigg|_{G_{r,p}=0}\right\} &= \operatorname{sign}\left\{\frac{\partial F_{reg}}{\partial [R]}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\}\right\} < 0, \\ \operatorname{sign}\left\{\frac{\partial G_{r,p}}{\partial x}\bigg|_{G_{r,p}=0}\right\} &= \operatorname{sign}\left\{\frac{\partial F_{reg}}{\partial [A]}([A], [R]); \{\{A\}_{c_A}, \{R\}_{c_R}\}\right\} > 0.\end{aligned}$$

□

S6. Elasticity

In this Section, the results linking the elasticity functions with perturbations (6) and (7) can be found as well as the elasticity estimates summarised in Table 1 in the main manuscript.

Lemma S4. *The sense of variation of the threshold function under the affinity perturbation (6) is determined by the elasticity function (8), by the expression*

$$\frac{\delta f_{m,l}}{\delta \eta}([A]; n) \bigg|_{\eta=1} = f_{m,l}([A]; n) (1 - \epsilon_{m,l}([A]; n)).$$

Proof. This result is a trivial consequence of Equation (S71) because that expression allows us to directly compute the variability of the threshold under perturbation (6)

$$\begin{aligned}\frac{\partial f_{m,l}}{\partial \eta}([A]; n) &= \frac{\partial}{\partial \eta} \left(\eta K_R \tilde{f}_{m,l} \left(\frac{[A]}{\eta K_A}; n \right) \right) \\ &= K_R \tilde{f}_{m,l} \left(\frac{[A]}{\eta K_A}; n \right) - \eta K_R \frac{[A]}{\eta^2 K_A} \tilde{f}'_{m,l} \left(\frac{[A]}{\eta K_A}; n \right)\end{aligned}$$

and consequently

$$\frac{\delta f_{m,l}}{\delta \eta}([A]; n) \bigg|_{\eta=1} = f_{m,l}([A]; n) - [A] f'_{m,l}([A]; n) = f_{m,l}([A]; n) (1 - \epsilon_{m,l}([A]; n)),$$

where we have used $f'_{m,l}([A]; n) = \frac{K_R}{K_A} \tilde{f}'_{m,l} \left(\frac{[A]}{K_A}; n \right)$. □

Corollary S1. *The thresholds determined by stimulated and recruitment BEWARE operators ($m = s/r$), (S15) and (S12), respectively, considering (S39)-(S45) ($l = t/p$) and assumption (S14), change in the same manner under affinity and enhancers' number reductions, that is (6) and (7) since:*

$$\begin{aligned}\operatorname{sign} \frac{\delta f_{m,l}}{\delta \eta}([A]; n) &= \operatorname{sign} \left(G_{m,l}(x, y; n-1) \bigg|_{x=\frac{[A]}{K_A}, y=\frac{f_{m,l}([A]; n)}{K_R}} \right), \\ &= \operatorname{sign} (f_{m,l}([A]; n-1) - f_{m,l}([A]; n)).\end{aligned}$$

Furthermore, in both cases, the limit behaviours they would tend to in the case of very low affinities ($\eta \rightarrow \infty$) and on the case of one only available enhancer coincides, that is,

$$\lim_{\eta \rightarrow \infty} f_{m,l}([A]; n) = \frac{\partial f_{m,l}}{\partial [A]}([A] = 0; n)[A] = f_{m,l}([A]; 1).$$

Proof. Combining Lemma S4 with expression (S79), we have that, for any BEWARE operator, it is verified

$$\begin{aligned}\frac{\delta f_{m,l}}{\delta \eta}([A]; n) &= \frac{1}{\eta} \left(\eta K_R \tilde{f}_{m,l} \left(\frac{[A]}{\eta K_A}; n \right) - [A] \frac{K_R}{K_A} \tilde{f}'_{m,l} \left(\frac{[A]}{\eta K_A}; n \right) \right) \\ &= \frac{1}{\eta \frac{\partial G_{m,l}}{\partial y}} \left(y \frac{\partial G_{m,l}}{\partial y}(x, y; n) + x \frac{\partial G_{m,l}}{\partial x}(x, y; n) \right) \Big|_{x=\frac{[A]}{\eta K_A}, y=\eta K_R \tilde{f}_{m,l} \left(\frac{[A]}{\eta K_A}; n \right)}\end{aligned}$$

which gives

$$\frac{\delta f_{m,l}}{\delta \eta}([A]; n) \Big|_{\eta=1} = \frac{1}{\frac{\partial G_{m,l}}{\partial y}} \left(y \frac{\partial G_{m,l}}{\partial y}(x, y; n) + x \frac{\partial G_{m,l}}{\partial x}(x, y; n) \right) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{m,l}([A]; n)}{K_R}} \quad (\text{S89})$$

Now, simple algebraic computations allow for rewriting this expression since

$$y \frac{\partial G_{m,l}}{\partial y}(x, y; n) + x \frac{\partial G_{m,l}}{\partial x}(x, y; n) = n G_{m,l}(x, y; n) - n G_{m,l}(x, y; n-1).$$

Let us remark that this expression is only valid in the stimulated approach if hypothesis (S14) is assumed.

The first identity of the statement of this Corollary can be trivially deduced from these expressions because

$$G_{m,l}(x, y; n) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{m,l}([A]; n)}{K_R}} = 0$$

holds by definition and, as mentioned in the proof of Proposition S2, it is also verified that:

$$\frac{\partial G_{m,l}}{\partial y}(x, y; n) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{m,l}([A]; n)}{K_R}} < 0.$$

The second identity is also obvious since

$$G_{m,l}(x, y; n-1) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{m,l}([A]; n)}{K_R}} > 0 \iff f_{m,l}([A]; n) < f_{m,l}([A]; n-1).$$

Let us now justify the thresholds limit in the case of very low affinity, that is, $\eta \rightarrow \infty$

$$\begin{aligned}\lim_{\eta \rightarrow \infty} f_{m,l}([A]; n) &= \lim_{\eta \rightarrow \infty} \eta \tilde{f}_{m,l} \left(\frac{[A]}{\eta K_A}; n \right) \\ &= \frac{K_R}{K_A} [A] \lim_{\eta \rightarrow \infty} \frac{\tilde{f}_{m,l} \left(\frac{[A]}{\eta K_A}; n \right) - \tilde{f}_{m,l}(0; n)}{\frac{[A]}{\eta K_A}} = \frac{K_R}{K_A} \tilde{f}'_{m,l}(0; n) [A].\end{aligned} \quad (\text{S90})$$

From (S79), we directly obtain $\tilde{f}'(0) = -\frac{\partial G}{\partial x}(0, 0)$. By a direct substitution of G function expressions for any BEWARE operator, we obtain

$$\tilde{f}'_{r,*}(0; n) = \frac{a-1}{1-r} \quad \tilde{f}'_{s,*}(0; n) = \frac{\nu_{max}^{(n)} \tilde{e}^{n-1}}{r_{bas}(1-\tilde{r})} = \frac{\nu_{max}^{(1)}}{\tilde{r}_{bas}(1-r)}$$

respectively for the recruitment and stimulated approaches. Substituting these expressions in (S90), we conclude the proof thanks to Remarks S6 and S7 in S5. \square

These formulas stated in this Corollary confirms that the reaction to perturbation (6) is strongly related with the variation of the same threshold under perturbation (7). Indeed, in all these expressions,

$$G_{m,l}(x, y; n-1) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{m,l}([A]; n)}{K_R}} \quad (\text{S91})$$

gives the value of the function G when the BEWARE operator has only $n - 1$ enhancers evaluated on the threshold obtained for the same BEWARE operator but with n enhancers. The sign of this computation is directly related to the relative positions of the thresholds for n or $n - 1$ enhancers because of (S77).

Our last Lemma is devoted to showing how different the predicted behaviours are to perturbations in affinity or the number of enhancers, that is, (6) or (7), depending on the stimulated or recruitment approach adopted to deduce the BEWARE operator. Our results suggest that, under the recruitment approach, the behaviour of the thresholds is determined basically by the kind of binding cooperativity relationships between the TFs, in the sense that, if some TF species cooperate only between them, then this is a competitive advantage that perturbations (6)–(7) interrupt. On the other side, the thresholds deduced from stimulated BEWARE operators are not only dependent on the cooperativity relations but also on the value of the parameter $\tilde{\epsilon}$. This, an a priori harmless parameter, has been proven to change the threshold elasticities provoking qualitative different responses, that is, it is able to alter the competitive advantages that cooperativities between TFs provide in an unclear way. The elasticity can be analytically estimated for thresholds deduced from BEWARE models for both stimulated and recruitment approaches in some cooperativity specific regimes: null/total cooperativity and in those cases when either only activators or only repressors can cooperate. All these estimates are collected in Table 1 in the main text.

Now, we focus on checking the elasticity estimates in Table 1 in the main text by considering separately the recruitment and stimulated approaches.

S6.1. Deduction of Elasticity Estimates in Table 1 (Main Manuscript): Recruitment Case

As we have shown in (S72), the threshold functions for the recruitment operator with total cooperativity are straight lines, independent from the number of enhancers n . Both facts allow us to deduce the unit elastic character of those thresholds.

Imposing $c_A = 1$ and $c_R > 1$ in definition (S75), we obtain that the implicit equation for the threshold $f_{r,p}(x; n)$ in the repressor cooperative case is

$$G_{r,p}(x, y; n) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{r,p}([A]; n)}{K_R}} = \bar{\alpha}_r^n - \alpha_r^n + (c_R - 1)(\gamma_r^n - \bar{\gamma}_r^n) = 0 \quad (\text{S92})$$

where $\bar{\alpha}_r$, α_r , γ_r and $\bar{\gamma}_r$ are evaluated at the points $\left(\frac{[A]}{K_A}, c_R \frac{f_{r,p}([A]; n)}{K_R}\right)$. Please note that, since $\gamma_r \geq \bar{\gamma}_r$ by definition, then $\alpha_r \geq \bar{\alpha}_r$ and we can order the functions such as

$$\alpha_r \geq \bar{\alpha}_r \geq \gamma_r \geq \bar{\gamma}_r. \quad (\text{S93})$$

The idea of the proof is to relate $G_{r,p}(\cdot, \cdot; n)$ with $G_{r,p}(\cdot, \cdot; n - 1)$ by considering the function

$$g(z) = z^{\frac{n}{n-1}}. \quad (\text{S94})$$

It is trivial to see that, by the mean value theorem,

$$\alpha_r^n - \bar{\alpha}_r^n = g(\alpha_r^{n-1}) - g(\bar{\alpha}_r^{n-1}) = g'(c)(\alpha_r^{n-1} - \bar{\alpha}_r^{n-1}) = \frac{n}{n-1} c^{\frac{1}{n-1}} (\alpha_r^{n-1} - \bar{\alpha}_r^{n-1}) \quad (\text{S95})$$

for a $c \in \mathbb{R}$ such as $\alpha_r^{n-1} > c > \bar{\alpha}_r^{n-1}$. The same goes for $\gamma_r^n - \bar{\gamma}_r^n$, where

$$\gamma_r^n - \bar{\gamma}_r^n = \frac{n}{n-1} \tilde{c}^{\frac{1}{n-1}} (\gamma_r^{n-1} - \bar{\gamma}_r^{n-1}) \quad (\text{S96})$$

for a $\tilde{c} \in \mathbb{R}$ such as $\gamma_r^{n-1} > \tilde{c} > \bar{\gamma}_r^{n-1}$. Obviously, inequalities (S93) imply $c > \tilde{c}$. Taking this relation into account, if we replace (S95)–(S96) in (S92), we obtain the following inequality

$$\frac{n}{n-1} c^{\frac{1}{n-1}} \left(\bar{\alpha}_r^{n-1} - \alpha_r^{n-1} + (c_R - 1)(\gamma_r^{n-1} - \bar{\gamma}_r^{n-1}) \right) > 0,$$

which implies

$$G_{r,p}(x, y; n-1) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{r,p}([A];n)}{K_R}} > 0$$

and hence the inelastic character ($\epsilon_{r,p} < 1$) of the threshold $f_{r,p}([A]; n)$ in the repressor cooperativity case by Lemma S4 and Corollary S1.

Analogously, we obtain in the opposite regime, $c_R = 1$ and $c_A > 1$, that

$$G_{r,p}(x, y; n) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{r,p}([A];n)}{K_R}} = \bar{\alpha}_r^n - \alpha_r^n + (c_A - 1)(\bar{\beta}_r^n - \beta_r^n) = 0$$

where now $\bar{\beta}_r < \beta_r < \alpha_r < \bar{\alpha}$ holds. Then, by using the function g in the same way, we can prove:

$$G_{r,p}(x, y; n-1) \Big|_{x=\frac{[A]}{K_A}, y=\frac{f_{r,p}([A];n)}{K_R}} = \bar{\alpha}_r^{n-1} - \alpha_r^{n-1} + (c_R - 1)(\bar{\gamma}_r^{n-1} - \gamma_r^{n-1}) < 0,$$

which, using Lemma S4 and Corollary S1, proves the elastic character ($\epsilon_{r,p} > 1$) of the threshold $f_{r,p}([A]; n)$ whenever $c_R = 1$ and $c_A > 1$.

S6.2. Deduction of Elasticity Estimates in Table 1 (Main Text), Stimulated Case ($n = 2$)

Recalling from (S7) that the threshold $f_{s,p}(x; 1)$ is a straight line of slope $m = \frac{v_{max}^{(1)}}{r_{bas}} \frac{1}{1-\bar{r}} \frac{K_R}{K_A}$, and evaluating the implicit threshold function (S87) on it, it is easy to check that

$$G_{s,p}(x, mx) = K_0 + K_1 x + K_2 x^2 \quad (S97)$$

with

$$K_i = \frac{2!}{i!(2-i)!} \left(r_{bas} \left((1+m\bar{r})^i - (1+m)^i + (c_A - 1) \left((\bar{r}m)^i - m^i \right) \right) + v_{max}^{(2)} \bar{e}^2 \left(\left(\frac{1}{\bar{e}} + m\bar{r} \right)^i - (m\bar{r})^i + (c_R - 1) \left(\frac{1}{\bar{e}} \right)^i \right) \right). \quad (S98)$$

In the null cooperativity case, $c_A = c_R = 1$, we obtain $K_0 = K_1 = 0$ and in consequence

- $G_{s,p}(x, mx) = 0$ and $\epsilon = 1 \forall x \geq 0 \iff K_2 = 0 \iff \frac{1}{\bar{e}} = \left(2 + \frac{v_{max}^{(1)}}{r_{bas}} \right) := t_1^{-1}$,
- $G_{s,p}(x, mx) < 0$ and $\epsilon < 1 \forall x \geq 0 \iff K_2 < 0 \iff \frac{1}{\bar{e}} < t_1^{-1}$,
- $G_{s,p}(x, mx) > 0$ and $\epsilon > 1 \forall x \geq 0 \iff K_2 > 0 \iff \frac{1}{\bar{e}} > t_1^{-1}$.

In the repressor cooperativity regime, $c_A = 1$ and $c_R > 1$, it can be estimated that $K_0 = 0$, $K_1 > 0$ and consequently

- $G_{s,p}(x, mx) > 0$ and $\epsilon > 1 \forall x \geq 0 \iff K_2 > 0 \iff \frac{c_R}{\bar{e}} > t_1^{-1}$.
- When $K_2 \leq 0$, which happens if and only if $\frac{c_R}{\bar{e}} \leq t_1^{-1}$, we obtain

$$- G_{s,p}(x, mx) \geq 0 \text{ and } \epsilon \geq 1 \text{ for } 0 \leq x \leq 2 \frac{c_R - 1}{\frac{1}{\bar{e}} - \frac{c_R}{\bar{e}}} := \frac{h_1}{K_A},$$

$$- G_{s,p}(x, mx) < 0 \text{ and } \epsilon < 1 \forall x > \frac{h_1}{K_A},$$

$t_1 = \left(2 + \frac{v_{max}^{(1)}}{r_{bas}} \right)^{-1}$ and $t_2 = \left(d2 + \frac{v_{max}^{(1)}}{r_{bas}} \frac{c_A(1+\bar{r})+2\bar{r}}{1-\bar{r}} \right)^{-1}$ being described in Table 1 in the main text.

Finally, in the case activators' cooperativity regime, $c_A > 1$ and $c_R = 1$, we obtain $K_0 = 0$, $K_1 < 0$ and consequently

- $G_{s,p}(x, mx) < 0$ and $\epsilon < 1 \forall x \geq 0 \iff K_2 < 0 \iff \frac{1}{\bar{e}} < 2 + \frac{c_A(\bar{r}+1)+2\bar{r}}{1-\bar{r}} \frac{v_{max}^{(1)}}{r_{bas}} := t_2^{-1}$.
- When $K_2 \geq 0$, which occurs if and only if $\frac{1}{\bar{e}} \geq t_2^{-1}$, then we have that

$$- G_{s,p}(x, mx) \leq 0 \text{ and } \epsilon \leq 1 \text{ for } 0 \leq x \leq 2 \frac{c_A - 1}{\frac{1}{\bar{e}} - \frac{1}{t_2}} := \frac{h_2}{K_A},$$

$$- G_{s,p}(x, mx) > 0 \text{ and } \epsilon > 1 \quad \forall x > \frac{h_2}{K_A}.$$

Let us mention that the proof of the total cooperativity case is analogous to the proof of the null cooperativity case.

In summary, the elasticities of the thresholds of the stimulated operator with two enhancers depend not only on the cooperativity considered, but also on the rest of the model parameters in terms of the quantities: $t_1 = \left(2 + \frac{v_{max}^{(1)}}{r_{bas}}\right)^{-1}$, $h_1 = 2K_A \frac{c_R - 1}{\frac{1}{t_1} - \frac{c_R}{\epsilon}}$, $t_2 = \left(2 + \frac{v_{max}^{(1)}}{r_{bas}} \frac{c_A(1+\tilde{r}) + 2\tilde{r}}{1-\tilde{r}}\right)^{-1}$, $h_2 = 2K_A \frac{c_A - 1}{\frac{1}{t_2} - \frac{1}{\epsilon}}$.

S7. Threshold Sensitivity Analysis with Hill Type Operators

Please note that the previous sensitivity analysis can be also applied in the Hill modelling framework. That is, making the reduction to the global Activation and Repressor variables,

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} F_{reg}([A], [R]; \{\{A, R\}_c\}) = \frac{1 + \left(a \frac{[A]}{K_d} + r \frac{[R]}{K_d}\right)^n}{1 + \left(\frac{[A]}{K_d} + \frac{[R]}{K_d}\right)^n}, \quad (S99)$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Basal([A], [R]; \{\{A, R\}_c\}) = \frac{1 + \left(\frac{[A]}{K_d} + \frac{[R]}{K_d}\right)^n}{1 + \left(\frac{[A]}{K_d} + \frac{[R]}{K_d}\right)^n}, \quad (S100)$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Promoter([A], [R]; \{\{A, R\}_c\}) = \frac{\left(\frac{[A]}{K_d} + \tilde{e}\tilde{r} \frac{[R]}{K_d}\right)^n - \left(\tilde{e}\tilde{r} \frac{[R]}{K_d}\right)^n}{1 + \left(\frac{[A]}{K_d} + \frac{[R]}{K_d}\right)^n},$$

and

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} F_{reg}([A], [R]; \{\{A\}_c, \{R\}_c\}) = \frac{1 + \left(a \frac{[A]}{K_d}\right)^n + \left(r \frac{[R]}{K_d}\right)^n}{1 + \left(\frac{[A]}{K_d}\right)^n + \left(\frac{[R]}{K_d}\right)^n}, \quad (S101)$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Basal([A], [R]; \{\{A\}_c, \{R\}_c\}) = \frac{1 + \left(\frac{[A]}{K_d}\right)^n + \left(\tilde{r} \frac{[R]}{K_d}\right)^n}{1 + \left(\frac{[A]}{K_d}\right)^n + \left(\frac{[R]}{K_d}\right)^n}, \quad (S102)$$

$$\lim_{\substack{c \rightarrow \infty \\ K_d = cte}} Promoter([A], [R]; \{\{A\}_c, \{R\}_c\}) = \frac{\left(\tilde{e}\tilde{r} \frac{[R]}{K_d}\right)^n}{1 + \left(\frac{[A]}{K_d}\right)^n + \left(\frac{[R]}{K_d}\right)^n}, \quad (S103)$$

where we can deduce the threshold implicit function

$$G_{*,*}^H(x, y; n) \Big|_{x=\frac{[A]}{K_d}, y=\frac{f_{*,*}^H([A]; n)}{K_d}} = 0, \quad (S104)$$

with $f_{*,*}^H([A]; n)$ the threshold function for the Hill modules. Moreover, Lemma S4 is also fulfilled in this case, where it is easy to check that all thresholds' functions are straight lines in the $[A] - [R]$ plane, and hence $\epsilon = 1$. In order to prove that $f_{*,*}^H([A])$ are linear for all the Hill BEWARE operators (S99)-(S103), we need to first obtain the implicit equation for each threshold. Following the same procedure done in the non-extreme cooperativity case, we obtain

$$G_{r,t}^H(x, y; n) = (ax + ry)^n - (x + y)^n, \quad (S105)$$

$$G_{s,t}^H(x, y; n) = r_{bas}((x + \tilde{r}y)^n - (x + y)^n) + v_{max}^{(n)}((x + \tilde{e}\tilde{r}y)^n - (\tilde{e}\tilde{r}y)^n), \quad (S106)$$

$$G_{r,p}^H(x, y; n) = (r^n - 1)y^n + (a^n - 1)x^n \quad (S107)$$

and

$$G_{s,p}^H(x, y; n) = r_{bas}(\tilde{r}^n - 1)y^n + v_{max}^{(n)}x^n. \quad (S108)$$

Since all these functions are homogeneous, that is, $G^H(x, y; n) = x^n G^H(1, y/x; n)$, it is straightforward to check that

$$\left(y \frac{\partial G_{*,*}^H}{\partial y}(x, y; n) + x \frac{\partial G_{*,*}^H}{\partial x}(x, y; n) \right) = n G_{*,*}^H(x, y; n)$$

which means, from Equation (S89), that the threshold is a straight line in the $[A] - [R]$ plane, and hence $\epsilon = 1$ from Lemma S4.

However, it is important to note that Collorary S1 is not fulfilled in general, and the dependence of $f_{*,*}^H([A]; n)$ with the number of enhancers varies depending on different parameter relations in the Hill versions of the Recruitment and Stimulated BEWARE operators.

S8. Parameters

Table S3. Parameters used in Figure 2 in the main manuscript for the Recruitment BEWARE operator with partial cooperativity.

Parameter	Value	Parameter	Value
C_B (nMmin ⁻¹)	1	r (a.u)	0.2
$K_{RP}/[RNAP]$ (a.u)	1	K_A (nM)	0.8
c_A (a.u)	25	K_R (nM)	0.8
c_R (a.u)	100	n (a.u)	3
a (a.u)	4.7		

Table S4. Parameters used in Figure 3 in the main text for the Stimulated BEWARE operator with null cooperativity under perturbations (6) and (7) in the main text. These parameters have been adopted from [16].

Parameter (present work)	Parameter (in [16])	$\epsilon > 1$	$\epsilon = 1$	$\epsilon < 1$
\tilde{e} (a.u)	$0.5\epsilon_A$	0.1	0.25	0.5
\tilde{r} (a.u)	$\rho = 0.5\epsilon_R$	0.5	0.5	0.5
r_{bas} (nMmin ⁻¹)	S_{XB}	50	50	50
$v_{max}^{(1)}$ (nMmin ⁻¹)	S_{XA}	100	100	100
K_A (nM)	$K_{A1} = K_{A2}$	4	4	4
K_R (nM)	$K_{R1} = K_{R2}$	1	1	1
c (a.u)	$c_A = c_R = c_{AR}$	1	1	1
$K_{RP}/[RNAP]$ (a.u)		1	1	1

Table S5. Parameters used in Figure S1 for the Recruitment BEWARE operator with total cooperativity (inverse logic case).

Parameter	(A)	(B)
C_B ($nMmin^{-1}$)	1	1
$K_{RP}/[RNAP]$ ($a.u$)	1	1
c ($a.u$)	400	400
a ($a.u$)	1.1	2.5
r ($a.u$)	0.1	0.9
K_A (nM)	20	100
K_R (nM)	100	20
n ($a.u$)	3	3

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