Generalized, switch-like competitive heterodimerization networks

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Abstract

High-dimensional switches have been proposed as a way to model cellular differentiation, particularly in the context of basic Helix-Loop-Helix (bHLH) competitive heterodimerization networks. A previous study derived a simple rule showing how many elements can be co-expressed, depending on the rate of competition within the network. A limitation to that rule, however, is that many biochemical parameters were considered to be identical. Here we derive a generalized rule. This in turns allows one to study more ways in which these networks could be regulated, linking intrinsic cellular differentiation determinants to extracellular cues.

1 Introduction

Switch-like responses are an essential aspect of the dynamics of signaling networks, and are expected to be crucial in mediating cellular differentiation, a process during which one cell-type is chosen and all others excluded, in an all-or-none fashion. Such responses have been documented experimentally (Xiong and Ferrell, 2003), and bistable switches have been thoroughly characterized from a mathematical point of view (Cherry and Adler, 2000). It is particularly noteworthy that in the course of cellular differentiation, many antagonistic genes are often co-expressed early-on despite their antagonism, before one gradually takes over (as discussed by Cinquin and Demongeot, 2005). bHLH proteins form a large family, which has been shown to have a crucial role in numerous instances of commitment to specific lineages and differentiation (Massari and Murre, 2000). It has been shown that models of bHLH networks can account particularly well for the co-expression of antagonistic genes early in the

differentiation process (Cinquin and Demongeot, 2005), although that study was limited to networks whose elements had identical biochemical parameters.

Here we generalize the study to a wider set of bHLH networks, and show that the simple rule which sets a limit to the number of genes which can be co-expressed, depending on the rate of competition in the network, still holds. The relaxed assumptions allow us to illustrate this rule in a context where differentiation outcome is specified by tuning the individual parameters of the elements in the network.

bHLH networks

Three important classes in the bHLH family, which are the basis for the mathematical model presented below, are the class A, ubiquitously-expressed transcriptional activators capable of forming homodimers and heterodimers, the class B, capable of providing promoter-specific transcriptional activation only when heterodimerized with a class A element, and Id proteins, which have been most often reported to form transcriptionally-unproductive heterodimers with the class A. Since different class B proteins bind the same class A partners, there can be some competition between them for access to those partners. In networks in which class B proteins auto-activate their own transcription (a common feature of determinants of cellular differentiation), class B proteins can therefore inhibit one another's expression, by titrating out the class A. Id proteins have the same effect of titrating out the class A, but are not explicitly taken into account in the model below because they have not been shown to regulate their own expression.

Regulation of differentiation

It has been proposed that the differentiation of some cell-types has a stochastic aspect, but in many instances, extra-cellular cues play an essential role in controlling cell-fate, although the details of the pathway from extra-cellular cue to intrinsic differentiation determinants are not always clear. Interestingly, the synthesis and degradation rates of key transcription factors have been shown in different instances to be regulated (see Ebert et al., 2003, Lim and Choi, 2004, zur Lage et al., 2004, for examples of regulated synthesis rates, and Horwitz, 1996, Trott et al., 2001, Sriuranpong et al., 2002, Viñals et al., 2004, for examples of regulated degradation rates). The activity of transcription factors can be directly regulated by post-translational modifications such as phosphorylation (for example phosphorylation of myogenic factors can decrease their activity, Winter et al., 1993, Zhou and Olson, 1994, Suelves et al., 2004, and this can also be the case for class A proteins, Page et al., 2004), by physical interactions with other proteins (Bengal et al., 1992, Perry et al., 2001), or indirectly by affecting cofactors (Simone et al., 2004, Seo et al., 2005). Phosphorylation can also modulate the propensity of bHLH proteins to form heterodimers (this can be the case for class B proteins, Firulli et al., 2003, class A proteins, Sloan et al., 1996, Lluis et al., 2005, and also Id proteins, Hara et al., 1997, Deed et al., 1997). It seems to generally be the case that upon cell differentiation, the activity of transcription factors associated with the cell-fate is enhanced.

Within the framework proposed here, the biasing of complex cell-fate decisions to specific outcomes can be mediated by the up- or down-regulation of synthesis rates or affinity for common class A activators, or down- or up-regulation of degradation rates, for class B genes associated to favored and unfavored outcomes, respectively. Different signaling pathways can act on one or many factors and do not need to directly cross-talk, as all the inputs are integrated by the competition between the switch elements.

The result of the decision can be regulated by the synthesis, class A-affinities, and degradation rates of the switch elements, while its timing is dependent on the level of competition

in the system: an increase in the competition level, which can for example be mediated by an increase in Id protein expression (shown in many experimental contexts, see references in Cinquin and Demongeot, 2005) sequestering the common activator away from all the elements of the switch, will force the weakest elements to be turned off. The efficiency with which Id proteins sequester the common activator can also be modulated by phosphorylation, which can also just be modeled by a change in the quantity of common activator available for switch elements. Some Id proteins, despite being paradoxically called "Inhibitors of differentiation", have indeed been shown recently to drive tumor-suppression and differentiation (Russell et al., 2004, Yu et al., 2005), as suggested by Cinquin and Demongeot (2005).

Mathematical model

A simple kind of model trying to account for switch-like behavior is where a set of class B proteins activate their own transcription. If class A proteins are considered to be expressed in a constitutive way, and not subjected to regulated degradation, they are present at a constant level. Only the time-evolution of each of the class B species is thus of interest. Calling $x_i,\ i = 1..n$, the concentrations of B_i (class B species), the equations are

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = -d_i x_i + \sigma_i \frac{x_i^2}{\alpha D^2 + x_i^2}, \tag{1}$$

with $D = 1 + \Sigma_{i=1}^{n} x_i$, $\alpha = K_2^2/a_t^2 \ \in \mathbb{R}_*^+$, where K_2 is the concentration of $A - B_i$ complex at which B_i transcription is half-maximal, a_t is the total quantity of class A proteins, σ_i and d_i are respectively the maximal synthesis rate and the degradation rate of B_i, and where each x_i is normalized with respect to the dissociation constant for the $A - B_i$ complex (this normalization leads to each maximal synthesis rate σ_i being divided by the dissociation constant of the $A - B_i$ complex, see Appendix A). The equations assume that for all i the quantity of $A - B_i$ complexes is negligible compared to the total quantity of B_i (see Cinquin, for a relaxation of that assumption).

This set of equations is the same as derived by Cinquin and Demongeot (2005), without the restriction $\forall\ i,\ d_i = 1,\ \sigma_i = \sigma$. We perform a steady state analysis of the system, assuming that it equilibrates over a time scale much shorter than that of cellular differentiation; this assumption is supported by the fact that transcription factors commonly have very short half-lives, which can be as low as a few minutes, while cellular differentiation often takes place over the course of hours or days.

Previous result

It was shown by Cinquin and Demongeot (2005) that, in the case where $\forall\ i,\ d_i = 1,\ \sigma_i = \sigma$ and $\sigma >> 1$, there are stable steady states with k elements "on" (*i.e.* non-0) if and only if

$$\alpha < 1/k^2$$

(when the condition $\sigma >> 1$ is not met, the above condition is necessary but not sufficient).

Since α is a measure of the harshness of the competition in the system (as it depends on the quantity of the common class A activators, and the heterodimer concentration giving half-maximal transcription), this shows that the harsher the competition in the system, the lower the number of elements which can co-exist.

2 Results

Let $r_i = \sigma_i / d_i$. Then at any stationary state,

$$\forall\ i \text{ st } x_i \neq 0,\ x_i^2 - r_i x_i + \alpha D^2 = 0,$$

and

$$\forall\ i \text{ st } x_i \neq 0,\ x_i = \frac{r_i \pm \sqrt{r_i^2 - 4\alpha D^2}}{2} \tag{2}$$

x_is at 0 can be discarded from the rest of the analysis. It will be shown below that if the stationary state is stable, at most one x_i can be at the lower solution of equation 2 (inequality 5). Suppose that there is such an x_κ (if there is not, a stronger inequality is derived, see Appendix B), and let κ' be such that $r_{\kappa'} = \max_i r_i$. It will be shown below that any steady state where $\kappa = \kappa'$ is unstable, and we can therefore suppose $\kappa \neq \kappa'$. Then

$$2\,(D-1) = \Sigma_{i\neq\kappa}\left(r_i + \sqrt{r_i^2 - 4\alpha D^2}\right) + r_\kappa - \sqrt{r_\kappa^2 - 4\alpha D^2}$$

$$\Sigma_i r_i + 2 = 2D - \Sigma_{i\neq\kappa}\sqrt{r_i^2 - 4\alpha D^2} + \sqrt{r_\kappa^2 - 4\alpha D^2}$$

$$\Sigma_i r_i + 2 \leq 2D - \Sigma_{i\neq\kappa,\ i\neq\kappa'}\sqrt{r_i^2 - 4\alpha D^2}$$

Consider the right-hand side of the above inequality as a function of D. It is an increasing function, and for the above inequality to hold, it must also hold for the maximum of that function (which is for $D = r_s/2\sqrt{\alpha}$), where $r_s = \min_i r_i$, ie

$$\frac{r_s}{\sqrt{\alpha}} \geq 2 + \Sigma_i r_i + \Sigma_{i\neq\kappa,\ i\neq\kappa'}\sqrt{r_i^2 - r_s^2} \tag{3}$$

This implies in particular $\alpha \leq 1/k^2$, where k is the number of non-zero x_is, generalizing the result obtained by Cinquin and Demongeot (2005).

2.1 Study of the characteristic polynomial

In a stable steady state at most one x_i takes the "lower" solution

For convenience, let $\beta_i = 1/r_i$. The Jacobian matrix of the system defined by equation 1, at a stationary point x in which none of the species has $x_i = 0$, is given by

$$J_{i,j}(x) = -P_i + \delta_{i,j} Q_i,\ i,j = 1..n$$

where $P_i = 2d_i\alpha D\beta_i$, and $Q_i = d_i\,(1 - 2\beta_i x_i)$. We assume that $\alpha > 0$ and note that $P_i > 0$. Eigenvalues λ of J are solutions to the equation $\det\,(J - \lambda I_n) = 0$.

$$\det\,(J-\lambda I_n) = \begin{vmatrix} Q_1 - P_1 - \lambda & -P_1 & -P_1 & \cdots & -P_1 \\ -P_2 & Q_2 - P_2 - \lambda & -P_2 & \cdots & -P_2 \\ \vdots & & & & \\ \vdots & & & & \\ -P_n & \cdots & \cdots & -P_n & Q_n - P_n - \lambda \end{vmatrix}$$

$$\det(J-\lambda I_n)=(\Pi_{i=1}^n P_i)\begin{vmatrix} \frac{Q_1}{P_1}-1-\frac{\lambda}{P_1} & -1 & -1 & \cdots & -1 \\ -1 & \frac{Q_2}{P_2}-1-\frac{\lambda}{P_2} & -1 & \cdots & -1 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & -1 \\ -1 & \cdots & \cdots & -1 & \frac{Q_n}{P_n}-1-\frac{\lambda}{P_n} \end{vmatrix}$$

$$\det(J-\lambda I_n)=(\Pi_{i=1}^n P_i)\begin{vmatrix} \frac{Q_1}{P_1}-1-\frac{\lambda}{P_1} & -1 & -1 & \cdots & -1 \\ -\frac{Q_1}{P_1}+\frac{\lambda}{P_1} & \frac{Q_2}{P_2}-\frac{\lambda}{P_2} & 0 & \cdots & 0 \\ \vdots & 0 & \ddots & \ddots & \vdots \\ \vdots & \vdots & \ddots & \ddots & 0 \\ -\frac{Q_1}{P_1}+\frac{\lambda}{P_1} & 0 & \cdots & 0 & \frac{Q_n}{P_n}-\frac{\lambda}{P_n} \end{vmatrix}$$

With $A=1-\frac{1}{\frac{Q_1}{P_1}-\frac{\lambda}{P_1}}$,

$$\det(J-\lambda I_n)=(\Pi_{i=1}^n P_i)\left(\frac{Q_1}{P_1}-\frac{\lambda}{P_1}\right)\begin{vmatrix} A & -1 & -1 & \cdots & -1 \\ -1 & \frac{Q_2}{P_2}-\frac{\lambda}{P_2} & 0 & \cdots & 0 \\ \vdots & 0 & \ddots & \ddots & \vdots \\ \vdots & \vdots & \ddots & \ddots & 0 \\ -1 & 0 & \cdots & 0 & \frac{Q_n}{P_n}-\frac{\lambda}{P_n} \end{vmatrix}$$

With $B_i=\frac{Q_i}{P_i}-\frac{\lambda}{P_i}$,

$$\det(J-\lambda I_n)=(\Pi_{i=1}^n P_i)\,B_1\begin{vmatrix} A & -1 & -1 & \cdots & -1 \\ -1 & B_2 & 0 & \cdots & 0 \\ \vdots & 0 & \ddots & \ddots & \vdots \\ \vdots & \vdots & \ddots & \ddots & 0 \\ -1 & 0 & \cdots & 0 & B_n \end{vmatrix}$$

For $n\geq 2$, let

$$L_n=\begin{vmatrix} A & -1 & \cdots & \cdots & -1 \\ -1 & B_2 & 0 & \cdots & 0 \\ -1 & 0 & B_3 & \cdots & 0 \\ \vdots & 0 & \cdots & \ddots & 0 \\ -1 & 0 & \cdots & 0 & B_n \end{vmatrix}$$

By developing with respect to the last column, $L_n=B_nL_{n-1}-(-1)^{n-1}C_{n-1}$, where

$$C_{n-1}=\begin{vmatrix} -1 & \cdots & \cdots & \cdots & -1 \\ B_2 & 0 & \cdots & \cdots & 0 \\ 0 & B_3 & 0 & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & 0 \\ 0 & \cdots & 0 & B_{n-1} & 0 \end{vmatrix}$$

By developing with respect to the last row, $C_{n-1}=-B_{n-1}C_{n-2}$ for $n\geq 4$. Since $C_2=\begin{vmatrix} -1 & -1 \\ B_2 & 0 \end{vmatrix}=B_2$, by induction $C_n=(-1)^n\,\Pi_{i=2}^n B_i$.

Therefore, $L_n = B_n L_{n-1} - \Pi_{i=2}^{n-1} B_i$ for $n \geq 3$. Since $L_2 = \begin{vmatrix} A & -1 \\ -1 & B_2 \end{vmatrix} = AB_2 - 1$, it can be shown by induction that

$$L_n = A\Pi_{i=2}^{n} B_i - \Sigma_{i=2}^{n} \Pi_{j=2, j\neq i}^{n} B_j, \text{ for } n \geq 2$$

Since $AB_1 = B_1 - 1$,

$$B_1 L_n = (B_1 - 1)\,\Pi_{i=2}^{n} B_i - B_1 \Sigma_{i=2}^{n} \Pi_{j=2, j\neq i}^{n} B_j = \Pi_{j=1}^{n} B_j - \Sigma_{i=1}^{n} \Pi_{j=1, j\neq i}^{n} B_j,$$

for $n \geq 2$.

Thus, $\det(J - \lambda I_n) = (\Pi_{i=1}^{n} P_i)\left(\Pi_{j=1}^{n} B_j - \Sigma_{i=1}^{n} \Pi_{j=1, j\neq i}^{n} B_j\right)$, and any eigenvalue λ of J satisfies

$$P(\lambda) = \Sigma_{i=1}^{n} \Pi_{j=1, j\neq i}^{n} B_j - \Pi_{j=1}^{n} B_j = 0, \tag{4}$$

with $B_i = \frac{Q_i}{P_i} - \frac{\lambda}{P_i}$. Suppose without loss of generality that Q_n and Q_{n-1} are respectively the largest and second-largest Q_i. Suppose in addition that these largest values are unique (the case where they are not will be dealt with below). Then $P(Q_n)$ has the same sign as $(-1)^{n-1}$, and $P(Q_{n-1})$ has the same sign as $(-1)^n$. Thus, $\exists\ t \in]Q_{n-1}, Q_n[$ s.t. $P(t) = 0$. Therefore, at any stable steady-state, $Q_{n-1} < 0$, and therefore $\forall\ i \neq n,\ Q_i < 0$, meaning

$$d_i\,(1 - 2\beta_i x_i) < 0$$

$$\forall\ i < n,\ x_i > \frac{1}{2\beta_i} = \frac{r_i}{2} \tag{5}$$

Therefore, at any stable steady-state, any x_i with $i < n$ is at the higher solution of equation 2, and

$$\forall\ i < n,\ x_i = \frac{r_i + \sqrt{r_i^2 - 4\alpha D^2}}{2}$$

If Q_n or Q_{n-1} are not unique in the re-numbering scheme discussed above, then the nonunique value is a root of P and hence cannot be positive. Therefore at most one Q_i can be positive and it is possible to renumber for a non-strict version of inequality 5 to hold. Strictness follows follows since $\alpha \neq 0$.

In a stable steady state, "$\kappa \neq \kappa'$"

We now show that there is no stable steady state with $r_n = \max_i r_i$. Suppose that $Q_n > 0$, $Q_{n-1} < 0$, and $r_n = \max_i r_i$. Then

$$P(0) = \Pi_{j\neq n} Q_j/P_j + Q_n/P_n \Sigma_{i=1}^{n-1} \Pi_{j=1, j\neq i}^{n-1} Q_j/P_j - \Pi_{j=1}^{n} Q_j/P_j$$

$$P(0) = \left(\Pi_{j\neq n} Q_j/P_j + Q_n/P_n \Pi_{j=1}^{n-2} Q_j/P_j\right) + Q_n/P_n \Sigma_{i=1}^{n-2} \Pi_{j=1, j\neq i}^{n-1} Q_j/P_j - \Pi_{j=1}^{n} Q_j/P_j$$

$$P(0) = (Q_{n-1}/P_{n-1} + Q_n/P_n)\,\Pi_{j=1}^{n-2} Q_j/P_j + Q_n/P_n \Sigma_{i=1}^{n-2} \Pi_{j=1, j\neq i}^{n-1} Q_j/P_j - \Pi_{j=1}^{n} Q_j/P_j$$

The last two terms in the sum both have the same sign as $(-1)^n$.

Now consider

$$\frac{Q_n}{P_n}\frac{P_{n-1}}{Q_{n-1}} = \frac{1-2\beta_n x_n}{1-2\beta_{n-1}x_{n-1}}\frac{\beta_{n-1}}{\beta_n}$$

$$\frac{Q_n}{P_n}\frac{P_{n-1}}{Q_{n-1}} = -\frac{\sqrt{1-4\alpha D^2\beta_n^2}}{\sqrt{1-4\alpha D^2\beta_{n-1}^2}}\frac{\beta_{n-1}}{\beta_n}$$

By hypothesis, $\frac{\beta_{n-1}}{\beta_n} \geq 1$, and thus

$$Q_n/P_n \geq |Q_{n-1}/P_{n-1}|$$

Therefore, $P(0)$ has the same sign as $(-1)^n$. Since $P(Q_n)$ has the same sign as $(-1)^{n-1}$, P has a positive root, and the steady state is unstable.

3 Discussion

The results above show that, in the case where degradation and normalized synthesis rates are allowed to be different for each element of the network, it becomes more difficult for the system to sustain the co-expression of many elements. Indeed, equation 3 implies that the weakest element (in terms of the ratio of the maximal synthesis rate to the product of the degradation rate and the dissociation constant for heterodimer formation with class A proteins) that is "on" cannot be much weaker than the other ones which are being co-expressed (an intuitive result, since a weak element would be too easily repressed by the other ones, and wouldn't stay "on" in their presence). In addition to that, the competition level α restricts the number of elements which can be co-expressed, in the same way as when degradation and normalized synthesis rates are all equal.

There is a great variety of ways in which a switch network can be led from a state of co-expression of all its elements to a state where only one is expressed, by changes in the competition level and in the synthesis and degradation rates. We show here two numerical simulations, to illustrate equation 3. In Figure 1, the competition level is increased, in a network in which elements have different normalized synthesis to degradation ratios; the weakest non-zero element is turned off every time the competition reaches a threshold. In Figure 2, the competition level is kept constant, but one element is made progressively stronger, and turns off all the other ones. Of course, the alteration of all parameters at the same time would be a plausible biological situation.

The networks studied here have been described in the context of class A and class B bHLH heterodimerization, but they could have a much wider relevance. Hox proteins, crucial determinants of tissue identity, have been shown to depend heavily on common binding partners of the PBC and Meis families (Mann and Affolter, 1998). A subfamily of bHLH-leucine zipper proteins shows tissue-specific expression, homo- and hetero-dimerization, and alternative splicing of dominant-negative forms (Kuiper et al., 2004). Myc and Max, which have opposite roles on cell growth and proliferation, form homodimers and heterodimers with Mad, with different affinities (Grinberg et al., 2004).

Networks in which each element needs to repress all others can easily be created with competition for a common heterodimerization partner, rather than active repression of all other elements. Networks including other forms of cross-repression and asymmetrical topologies would also be of interest to study cellular differentiation, and are currently under investigation.

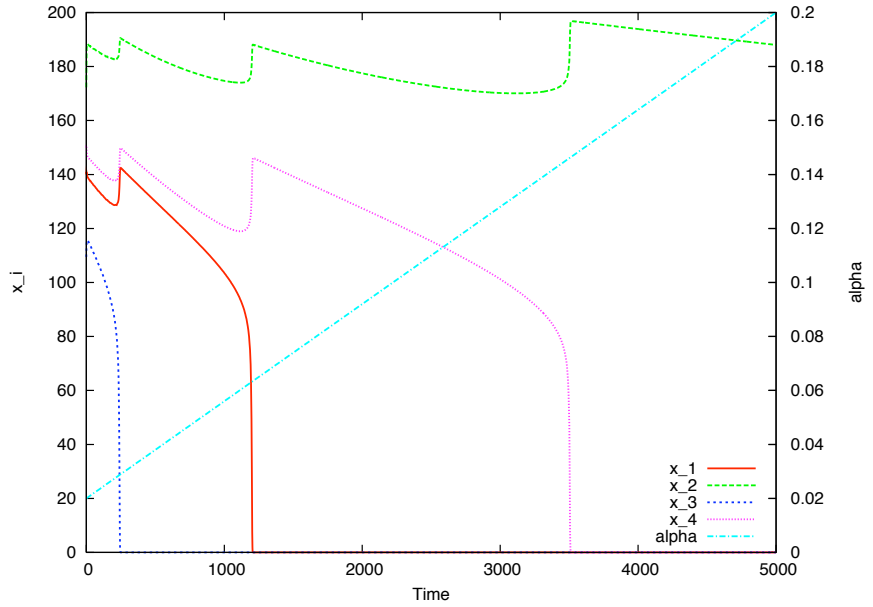

Figure 1: Simulation of a 4-dimensional switch defined by equations 1; the competition parameter α is progressively increased, causing the weakest non-0 element to be switched off periodically. Specific parameters are $d_i = 1$ for all i, $\sigma_1 = 190$, $\sigma_2 = 226$, $\sigma_3 = 177$, and $\sigma_4 = 195$.

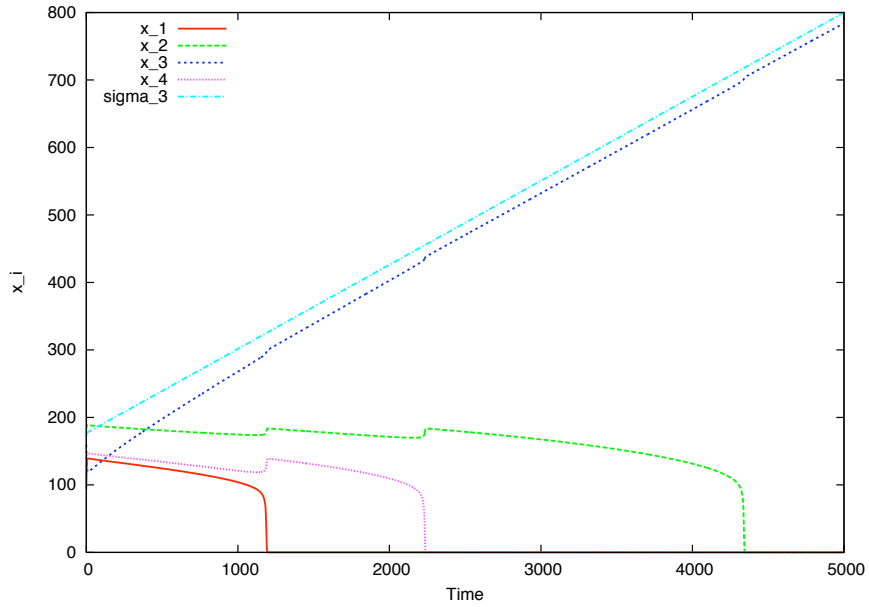

Figure 2: Simulation of a 4-dimensional switch defined by equations 1; the synthesis rate for x_3 is progressively increased, causing all other elements to be successively switched off. Other synthesis and degradation rates are as in Figure 1, and the competition rate $\alpha = 0.02$.

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A Normalization with respect to the $A - B_i$ dissociation constants

Supposing the $A-B_i$ dimerization reactions are at equilibrium (a reasonable assumption given the generally-fast rate of protein association by random 3D diffusion), and that $a_t \ll D_i$ for all i (see Cinquin, for a relaxation of that assumption), using the law of mass action one gets

$$[AB_i] = \frac{a_t[B_i]/D_i}{1 + \Sigma_j[B_j]/D_j},$$

where D_i is the dissociation constant for each $A-B_i$ complex, and a_t is the total quantity of A. If the synthesis of B_i depends on the concentration of the $A - B_i$ complex, in a non-linear fashion described by a Hill function of degree 2 with maximal value σ_i and half-maximal synthesis for $[AB_i] = K_2$, and B_i has a degradation rate d_i, writing $x_i = [B_i]$ one gets

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = -d_i x_i + \sigma_i \frac{[AB_i]^2}{K_2^2 + [AB_i]^2}$$

Now let $y_i = x_i/D_i$. Then

$$\frac{\mathrm{d}y_i}{\mathrm{d}t} = \frac{1}{D_i}\frac{\mathrm{d}x_i}{\mathrm{d}t} = -d_i y_i + \frac{\sigma_i}{D_i}\frac{y_i^2}{\alpha D^2 + y_i^2},$$

with $D = 1 + \Sigma_{i=1}^n y_i$, $\alpha = K_2^2/a_t^2 \ \in \mathbb{R}_*^+$.

The normalization with respect to the dissociation constants D_i has thus led to the replacement of each maximal synthesis rate σ_i by σ_i/D_i.

B Stronger inequality when no x_i is at the "lower solution"

If all x_is are given by the higher root of equation 2, one gets

$$2\,(D-1) = \Sigma_i r_i + \sqrt{r_i^2 - 4\alpha D^2}$$

$$\Sigma_i r_i + 2 = 2D - \Sigma_i \sqrt{r_i^2 - 4\alpha D^2}$$

With the same argument as previously,

$$\frac{r_s}{\sqrt{\alpha}} \geq 2 + \Sigma_i r_i + \Sigma_i \sqrt{r_i^2 - r_s^2}, \tag{6}$$

with $r_s = \min_i r_i$.