Introduction

The rate at which eukaryotic DNA is transcribed into mRNA is regulated largely by transcription factors (TFs) that bind to the cis-regulatory elements of genes and affect the affinity of RNA polymerase (RNAP) for the transcription initiation site of the gene. Differential gene expression is a direct result of different TF distributions in different cell types within the same organism, and is at the heart of differentiation, development, and evolution (see (Davidson 2001) for a review). Genes and TFs, together with the machinery for transcription, translation, transport, and inter-cellular signalling, form genetic regulatory networks (GRNs) that control gene expression.

GRNs can be modelled as series of coupled differential equations, whose (numerical) solution requires input of values for all parameters. Usually, however, few, if any, of these values are known. Although it is possible, at least in theory, to find the ‘best’ set of parameter values by sampling the whole of parameter space, many degenerate solutions may be expected (cf. (Von Dassow et al. 2000)). This is partly due to correlation between parameters, but it also occurs because biological systems have built-in regulation mechanisms that make them robust to changes in many of their parameter values. Therefore, most attempts to analyse the behaviour of GRNs, and large biological systems in general,
focus on simplifying the overall structure, whilst keeping the control elements intact (Kauffman 1993; Plahte et al. 1998; Thomas et al. 1995).

Several authors have pointed out that there are considerable similarities between biological and electronic control mechanisms, and tried to analyse, even design, biological networks using techniques that were developed originally for electronic circuits (Cherry and Adler 2000; McAdams and Shapiro 1995). Many TFs form dimers or higher-order oligomers, so that their binding to DNA is sigmiodally dependent on their concentration (Chenenov et al. 2000; Cicero et al. 2001; Latchman 1997; Millevoi et al. 2001) and references therein). The occurrence of S-shaped dependencies in all but the simplest of biological pathways has led to the conjecture that some processes may be idealised and modelled using digital switches (see e.g. (Kauffman 1993; Wolf and Eeckman 1998). Digital switches are characterised by the fact that they can be in two states only (off/on), and, if necessary, their dynamics are conveniently described using propagation delays (the time between the signal appearing at the input and the corresponding response at the output). Importantly, digital switches can be combined to form logic gates – modules performing logical operations such as AND or NOT, which, in turn, can be used in more complex switching circuits.

However, purely Boolean representations have lower predictive power than their continuous counterparts (reviewed in (Smolen et al. 2000)). Transcription rates may be anywhere on a continuous scale between 0 and maximal, and this can have important consequences for the rate at which other genes are transcribed, and hence for the dynamics of the network.

GRNs have also been modelled as ‘classical’ or ‘first order’ neural networks (Reinitz et al. 1995, Vohradsky 2001). In the neural network approach, the genes form the nodes of the network. The connections between the nodes have weights, which relate to the magnitude of the effect that a gene at one end of the connection exerts on the gene at the other end. Weights can be zero (no interaction), positive (stimulation of gene expression), or negative (repression). The effects are assumed to be additive so that transcription levels are continuously valued. However, this approach ignores the fact that there is often significant synergism - defined as deviation from additive behaviour - in the effect of multiple TFs on the expression of a single gene (see (Carey 1998; Herschlag and Johnson 1993; Latchman 1997; Salvador 2000) for reviews). Thus, the classical ANN approach cannot be used to describe the ubiquitous ‘AND’ interaction, in which individual transcription factors have no effect, but their combination does.

In an attempt to avoid the limitations associated with the Boolean and first order neural network approaches, Yuh et al. (Yuh et al. 1998; Yuh et al. 2001) used a combination of digital and analogue representations to model the regulation of the sea urchin embryo Endo16 gene. The expression of the endo16 gene is affected by no fewer than nine sequence-specific DNA binding factors, some of which modulate each other’s effect. The authors predicted (Yuh et al. 1998) and later demonstrated experimentally (Yuh et al. 2001) the existence of a cis-regulatory switch by which spatial control of the endo16 gene shifts during development.

Formally, in the approach introduced in the above studies, genes are modelled as so-called Sigma-Pi units. Sigma-Pi units were introduced as nodes in ‘higher-order’ neural networks by Rumelhart and McClelland (1986), in order to circumvent linear separability constraints associated with first-order neural networks. In a higher order network, a combination of inputs into the same node may generate an output that is different from their simple sum. Significantly, Boolean functions and logic gates can be expressed in the Sigma-Pi formalism, but the input to and output of a Sigma-Pi function is not restricted to Boolean values (Gurney 1997). Like logic gates, Sigma-Pi units are combinatorial, and consequently complex units may sometimes be decomposed into a set of simpler modules.

Central to the approach advocated by Yuh et al. is, as in electronic design, a circuit diagram, which describes the connectivity and overall organisation of the network. The attraction is not only that the interactions between TFs are visualised in a simple, modular way, but also that the circuit diagram can be used as a basis for simulation models. However, the authors did not describe the precise relationship between TF binding, gene expression, and combinatorial or sequential logic, nor have the assumptions on which the relationship is based been made explicit.

TF binding and dissociation are likely to be orders of magnitude faster than transcription and translation themselves, so that simple equilibrium thermodynamics can be used to calculate TF binding site occupancy. In this paper, we show how TF binding thermodynamics, gene activation, and cis-
regulatory logic are related, and we indicate how this approach may be used to simplify simulations and derive topologies of multi-gene networks.

Theory

Network dynamics: TF production

The rate at which the concentration of a protein changes inside a cell depends on the rate at which its mRNA is produced and degraded, on the rates at which the mRNA molecules are translated and, if necessary, the products are post-translationally modified, and on the rate at which the protein itself degrades. We assume that other processes, such as diffusion and transport are fast with respect to transcription and translation, and may be ignored. Thus, the rate of change in \( m \), the concentration of a specific mRNA, \( M \), and \( p \), the concentration of the translated product \( P \), are:

\[
\frac{dm}{dt} = k_{+1} - k_{-1}m 
\]

(1a)

\[
\frac{dp}{dt} = k_{+2}m - k_{-2}p
\]

(1b)

Here, \( k_{+1} \) is the rate at which \( M \) is produced, \( k_{-1} \) and \( k_{-2} \) are mRNA and protein degradation rates, and \( k_{+2} \) is the average translation rate.

If necessary – and justified – these equations can be simplified by assuming that the system rapidly relaxes to steady state, where \( \frac{dm}{dt} \) and \( \frac{dp}{dt} \) are zero, so that \( m = k_{+1}/k_{-1} \) and \( p = k_{+2}/k_{-1}k_{-2} \). However, the relaxation rates (the rates at which the system progresses toward steady state) are determined by \( k_{-1} \) and \( k_{-2} \). As protein lifetimes can be hours or days, the full steady state assumption will rarely be justified. However, mRNA often has a turnover rate of minutes. In that case, equation 1 and 2 may be combined to give

\[
\frac{dp}{dt} = k_{+1}k_{+2}/k_{-2} - k_{-2}p
\]

(1c)

In the following, we shall assume that the apparatus for mRNA breakdown, protein translation and modification, and protein degradation are in place and invariant, so that \( k_{-1} \), \( k_{-2} \), and \( k_{+2} \) are constants.

In the transcriptional networks that we aim to model, the mRNA transcription rate \( (k_{+1}) \) is dependent on the availability of particular proteins (TFs), whose concentrations are, in turn, determined by the availability of mRNA.

Network nodes: gene transcription

The transcription rate of a gene, \( k_{+1} \), is determined by \( k_{\text{ini}} \), the transcription initiation rate, and \( k_{\text{elong}} \), the transcription elongation rate. The value of \( k_{\text{ini}} \) is dependent on the presence or absence of transcription factors and other regulatory proteins in the ‘enhanceosome’, the TF/enhancer DNA complex (Carey 1998; Merika and Thanos 2001). The value of \( k_{\text{elong}} \) and the maximum value of \( k_{\text{ini}}, k_{\text{ini}}^{\text{max}} \), depend on factors such as the degree to which the gene is embedded in chromatin, and on the type of RNA polymerase (RNAp) that catalyses the transcription. The value of \( k_{\text{ini}}^{\text{max}} \) is likely to be different for different genes, but is constant for a single gene. Neither \( k_{\text{elong}} \), nor \( k_{\text{ini}}^{\text{max}} \) depend on TF concentrations.

The overall transcription rate \( k_{+1} \) is equal to \( k_{\text{ini}} \times k_{\text{elong}} \). The initiation rate \( k_{\text{ini}} \) is modulated by the enhanceosome. We define the relative activation factor, \( \phi \), as \( \phi = k_{\text{ini}} / k_{\text{ini}}^{\text{max}} \), so that the expression for \( k_{+1} \) becomes:

\[
k_{+1} = \phi \times k_{\text{ini}}^{\text{max}} \times k_{\text{elong}}
\]

(2)

Synergism

Different TF/DNA complexes modulate the rate by different factors. For example, TF \( T_A \) may individually give rise to a transcription rate of 0.1 times the maximum, and TF \( T_B \) may, by itself, not stimulate transcription at all, but together they may induce transcription at the maximum rate. Another TF, \( T_C \), may repress transcription induced by \( T_A \) and \( T_B \), but stimulate the transcription induced by \( T_B \).
alone. Thus, T_A, T_B, and T_C act synergistically to induce or repress gene transcription: their combined effect is different from the simple sum of their individual effects.

Synergistic action of TFs may have two causes. It may be due to cooperative effects in the binding of the TFs (if T_A is bound, binding of T_B is facilitated or made more difficult, and vice versa). Alternatively, the combination of T_A and T_B may form a more (or less) effective platform for RNAp assembly than T_A and T_B by themselves. Thus, synergism may be observed even if T_A and T_B bind non-cooperatively.

In the following, we shall assume that: a) a TF must be bound to its cognate site on the cis-regulatory domain before it can affect RNAp assembly, and b) TF binding occurs independently of RNAp assembly. Therefore, we shall describe the formation of DNA-TF complexes first, and then combine TF binding with the promotion of RNAp assembly.

Transcription factor binding

To illustrate the meaning of the symbols used here, we introduce as an example an equilibrium system, consisting of a substrate S (read: DNA) with a binding site S_A (read: cis-regulatory site) for a ligand L_A (read: a trans-regulatory element, i.e. TFs). We assume that the system is at equilibrium, and that the concentration of unbound L_A, a, is much larger than that of S_A, upon binding of L_A to S_A, a is effectively unchanged. If there are many molecules of S, the fraction y_A of all S molecules that have bound L_A, termed fractional saturation, is given by:

$$y_A = \frac{a}{K_A}$$

(3a)

Here, K_A is the (equilibrium) dissociation constant. Expression 3 is derived from the following equations:

$$K_A = \frac{[S_A]a}{[S-L_A]} \text{ (definition of } K_A)$$

(3b)

$$[S_A] + [S-L_A] = C \text{ (constant, mass conservation expression)}$$

(3c)

$$y_A = \frac{[S_A]}{C} \text{ (definition of fractional saturation)}$$

(3d)

$$a - C \approx a \text{ (excess ligand assumption)}$$

(3e)

K_A relates to the free energy of complex formation according to:

$$K_A = e^{\Delta G_A/RT}$$

(4)

in which \(\Delta G_A\) is the free energy difference between free S and the S-L_A complex, and R and T are the gas constant and the absolute temperature (the more familiar expression with a minus sign in the exponent relates to the equilibrium association constant, the inverse of \(K_A\)). K_A is measured in concentration units (i.e. in the same units as \(a\)), and is equal to the concentration of L_A at which half of all binding sites S_A are filled. Inside a cell there are of course only a few, or even just one, cis-regulatory binding sites for each trans-regulatory factor. Nonetheless, equation 3 is still valid, and is now interpreted as the probability that L_A is bound to S, and K_A is equal to the concentration of L_A at which S_A is filled for 50% of the time, and free for the other 50%.

We now introduce another binding site, S_B, where ligand L_B, whose concentration is b, binds with a dissociation constant K_B. If L_A and L_B bind independently (for instance because their binding sites are far apart), the dissociation constant for L_B will be the same in the absence and presence of bound L_A. However, if the presence of bound L_A affects the binding of L_B in any way, L_A and L_B are said to be binding cooperatively. In that case, K_B, the dissociation constant of L_B in the absence of bound L_A is different from \(K_{B(A)}\), the dissociation constant of L_B in the presence of bound L_A. The expression for the probability that both ligands are simultaneously bound is:

$$y_{AB} = \frac{ab / K_A K_{B(A)}}{1 + a / K_A + b / K_B + ab / K_A K_{B(A)}}$$

(5a)

Equation 5, as equation 3, is derived from the definitions of the equilibrium constants, the mass conservation requirement, and the assumption that the ligands are present in much higher
concentrations than the substrate. The free energy difference between the complex S-L\textsubscript{A}L\textsubscript{B} and free S, \(\Delta G_{\text{AB}}\), is the sum of \(\Delta G_A\) and the free energy difference between the complexes S-L\textsubscript{A} and S-L\textsubscript{A}L\textsubscript{B} (\(\Delta G_{\text{B}(A)} = \Delta G_B - \Delta G_A\)). The same expression holds for \(\Delta G_B\) and \(\Delta G_{\text{A}(B)}\), so that:

\[
\Delta G_A + \Delta G_{\text{B}(A)} = \Delta G_B + \Delta G_{\text{A}(B)}
\]

and the consequence of this is that:

\[
K_A \times K_{\text{B}(A)} = K_B \times K_{\text{A}(B)}
\]

Thus, equation 5 may also be written as

\[
y_{\text{AB}} = \frac{e^{\Delta G_{\text{AB}}/RT}ab}{1 + e^{\Delta G_A/RT}a + e^{\Delta G_B/RT}b + e^{\Delta G_{\text{AB}}/RT}ab}
\]

(5b)

and describes the partitioning of ‘state’ S-L\textsubscript{A}L\textsubscript{B} over the total number of possible states at the given concentrations of L\textsubscript{A} and L\textsubscript{B}, summed up in the denominator \(D\). Likewise, the fraction of S-L\textsubscript{A} (and not counting S-L\textsubscript{A}L\textsubscript{B}) is:

\[
y_{\text{A}} = \frac{e^{\Delta G_A/RT}a}{D}
\]

(5c)

and the fraction of S that has no ligand bound at all is:

\[
y_0 = \frac{1}{D}
\]

(5d)

By writing \(\alpha = a/K_A, \beta = b/K_B,\) and \(r_{\text{AB}} = K_{\text{B}(A)}/K_A\) the appearance of equations 5a, c, and d can be simplified:

\[
y_{\text{AB}} = \frac{r_{\text{AB}} \alpha \beta}{1 + \alpha + \beta + r_{\text{AB}} \alpha \beta}
\]

(5e)

\[
y_{\text{A}} = \frac{\alpha}{1 + \alpha + \beta + r_{\text{AB}} \alpha \beta}
\]

(5f)

and

\[
y_0 = \frac{1}{1 + \alpha + \beta + r_{\text{AB}} \alpha \beta}
\]

(5g)

Note that \(r_{\text{AB}}\) is a measure for the cooperativity in the binding of L\textsubscript{A} and L\textsubscript{B} to their respective binding sites on S, because:

\[
r_{\text{AB}} = e^{\Delta G_{\text{B}(A)} - \Delta G_A} = e^{\Delta G_{\text{A}(B)} - \Delta G_B}
\]

(6)

The same procedure can be followed in the derivation of the partitioning of three ligands L\textsubscript{A}, L\textsubscript{B}, and L\textsubscript{C}, with concentrations \(a, b,\) and \(c,\) binding constants \(K_A, K_B,\) and \(K_C,\) and ‘normalised’ concentrations \(\alpha, \beta,\) and \(\gamma\) (where \(\alpha = a/K_A, \) etc.).

\[
y_{\text{ABC}} = \frac{r_{\text{ABC}} \alpha \beta \gamma}{1 + \alpha + \beta + \gamma + r_{\text{AB}} \alpha \beta + r_{\text{AC}} \alpha \gamma + r_{\text{BC}} \beta \gamma + r_{\text{ABC}} \alpha \beta \gamma}
\]

(7a)

Here, \(r_{\text{ABC}} = r_{\text{A}(B)(C)} = r_{\text{A}(C)(B)} = r_{\text{C}(A)(B)}\), where \(r_{\text{A}(B)(C)} = K_{\text{D}(A)(B)(C)}\), the ratio of the dissociation constants of L\textsubscript{A} in the absence and presence of bound L\textsubscript{B} and L\textsubscript{C}. The fractional saturation \(A\) of the L\textsubscript{A} binding site is:

\[
A = y_A + y_{\text{AB}} + y_{\text{ABC}} = \frac{\alpha + r_{\text{AB}} \alpha \beta + r_{\text{AC}} \alpha \gamma + r_{\text{ABC}} \alpha \beta \gamma}{1 + \alpha + \beta + \gamma + r_{\text{AB}} \alpha \beta + r_{\text{AC}} \alpha \gamma + r_{\text{BC}} \beta \gamma + r_{\text{ABC}} \alpha \beta \gamma}
\]

(7b)

Note that if there is only one ligand, \(A\), the fractional saturation of the binding site for L\textsubscript{A}, is equal to \(y_A\), the probability of finding the complex. With more than one ligand, \(y_A\) and \(A\) are not the same.
Promotion of RNAp assembly

Each one of the possible complexes that are formed upon binding of a TF to its cognate region on cis-regulatory DNA affects to a certain extent the rate at which the full transcription machinery is assembled and put into operation. In the following we shall assume that the RNA polymerase (RNAp) ‘binding site’ is emptied immediately after RNAp has been fully assembled, because RNAp moves away from the initiation site during the transcription process. In contrast to TF binding, therefore, RNAp binding is not saturable. The extent to which each one of the TF-DNA complexes modulates the RNAp assembly rate is described by their individual modulation coefficients \( w \). One of the possible TF-DNA complexes will form the most effective platform, and we shall give this complex a modulation coefficient of 1.

Thus, if two TFs have individual modulation coefficientss \( w_A = 0.1 \), and \( w_B = 0.2 \) with respect to the modulation coefficient of the complex in which both are present (\( w_{AB} = 1 \)), the overall modulation factor \( \mu \) is:

\[
\varphi = \frac{0 \times 1}{1 + \alpha + \beta + r_{AB} \alpha \beta} + \frac{0.1 \alpha}{1 + \alpha + \beta + r_{AB} \alpha \beta} + \frac{0.2 \beta}{1 + \alpha + \beta + r_{AB} \alpha \beta} + \frac{r_{AB} \alpha \beta}{1 + \alpha + \beta + r_{AB} \alpha \beta}
\]

More general:

\[
\varphi = \frac{w_0 + w_A \alpha + w_B \beta + w_{AB} r_{AB} \alpha \beta}{1 + \alpha + \beta + r_{AB} \alpha \beta}
\]

(8a)

By definition, all modulation coefficients have values between 0 and 1, and the value of at least one of them must be 1. Thus, when in the above example, the \( L_A \) and \( L_B \) concentrations are sufficiently large, \( \mu \) approaches 1.

The general equation for \( \mu \) for three TFs is:

\[
\varphi = \frac{w_0 + w_A \alpha + w_B \beta + w_C \gamma + w_{AB} r_{AB} \alpha \beta + w_{AC} r_{AC} \alpha \gamma + w_{BC} r_{BC} \beta \gamma + w_{ABC} r_{ABC} \alpha \beta \gamma}{1 + \alpha + \beta + \gamma + r_{AB} \alpha \beta + r_{AC} \alpha \gamma + r_{BC} \beta \gamma + r_{ABC} \alpha \beta \gamma}
\]

(8b)

It can be intuitively understood that it is possible to further generalise this treatment. The expression for activation by \( N \) ligands is given in Appendix 1.

Thus, by assuming that TF binding and RNAp assembly are fast with respect to the rate at which the transcription product is produced, the instantaneous mRNA production rate \( k_{\text{ini}} \) (eqn 1a or 1c) can be estimated from the values of \( k_{\text{ini}} \), \( k_{\text{elong}} \), the (normalised) concentrations of the TFs involved in the transcription initiation, the degree of cooperativity in the binding of the various TFs, and the modulation coefficients for the individual complexes (eqn 2, combined with eqns 8 or A1).

Modules

Cis-regulatory DNA may consist of several distinct domains or modules. Modules may have evolved to regulate gene expression in different development stages of the organism, and act relatively autonomously. The interaction between individual modules within a complete cis-regulatory domain is likely to be minimal, albeit not necessarily zero.

Consider, for instance, a case in which three TFs regulate gene expression. The binding sites for \( L_A \) and \( L_B \) form one module, the binding site for \( L_C \) forms another, and both act completely autonomously. Furthermore, free DNA (no TFs bound) does not promote assembly of RNAp at all. This means that binding of \( L_C \) is independent of that of \( L_A \) and \( L_B \), and that bound \( L_C \) forms the same platform for RNAp assembly in the presence and absence of bound \( L_A \) or \( L_B \). In other words, in this example \( w_B = 0 \), \( r_{AC} = r_{BC} = 1 \), \( r_{ABC} = r_{AB} \), and \( w_{AC} = w_A + w_C \), \( w_{BC} = w_B + w_C \), and \( w_{ABC} = w_{AB} + w_C \). Thus, equation 8b simplifies to an expression with far fewer parameters, in which the modular nature of the complex is apparent:
Thus, in this example, $\mu$ has two distinguishable contributions: one from the module formed by the L$_A$ and L$_B$ binding sites, and a second from the module formed by the L$_C$ binding site. In order to rewrite this and similar equations in a more tractable form, we need to look at and rewrite the equations for non-cooperative ligand binding first.

**Non-cooperative TF binding**

If, in general, two or more TFs bind completely independently (non-cooperatively), all values of $r$ are 1, and the denominators in equations 5 (two TFs, $D_2$) and 7 (three TFs, $D_3$) can be factorised as:

$$D_2 = 1 + \alpha + \beta + \alpha\beta = (1 + \alpha)(1 + \beta) \quad (9a)$$

$$D_3 = 1 + \alpha + \beta + \gamma + \alpha\beta + \alpha\gamma + \beta\gamma + \alpha\beta\gamma = (1 + \alpha)(1 + \beta)(1 + \gamma) \quad (9b)$$

which can be extended way to any number of TFs. The expression for $\varphi_2$ (two TFs) and $\varphi_3$ (three TFs) become:

$$\varphi_2 = \frac{w_0 + w_\alpha + w_\beta + w_{\alpha\beta}}{(1 + \alpha)(1 + \beta)} \quad (9c)$$

$$\varphi_3 = \frac{w_0 + w_\alpha + w_\beta + w_\gamma + w_{\alpha\beta} + w_{\alpha\gamma} + w_{\beta\gamma} + w_{\alpha\beta\gamma}}{(1 + \alpha)(1 + \beta)(1 + \gamma)} \quad (9d)$$

If we now substitute $\alpha/(1 + \alpha)$ with $A$, $\beta/(1 + \beta)$ with $B$, and $\gamma/(1 + \gamma)$ with $C$, equations 9c and d are written as:

$$\varphi_2 = w_0(1 - A)(1 - B) + w_\alpha A(1 - B) + w_\beta (1 - A)B + w_{\alpha\beta} AB =$$

$$= w_0 + (w_\alpha - w_0)A + (w_\beta - w_0)B + (w_{\alpha\beta} - (w_\alpha + w_\beta) + w_0)AB =$$

$$= W_0 + W_\alpha A + W_\beta B + W_{\alpha\beta} AB \quad (9e)$$

and:

$$\varphi_3 = W_0 + W_\alpha A + W_\beta B + W_\gamma C + W_{\alpha\beta} AB + W_{\alpha\gamma} AC + W_{\beta\gamma} BC + W_{\alpha\beta\gamma} ABC \quad (9f)$$

in which:

$$W_0 = w_0 \quad (9g)$$

$$W_\alpha = w_\alpha - w_0, \text{ etc.} \quad (9h)$$

$$W_{\alpha\beta} = w_{\alpha\beta} - (w_\alpha + w_\beta) + w_0, \text{ etc.} \quad (9i)$$

$$W_{\alpha\beta\gamma} = w_{\alpha\beta\gamma} - (w_{\alpha\beta} + w_{\alpha\gamma} + w_{\beta\gamma}) + (w_\alpha + w_\beta + w_\gamma) - w_0 \quad (9j)$$

In these equations, $A$, $B$, and $C$ are the fractional saturations of the binding sites for L$_A$, L$_B$, and L$_C$ (see eqn 7c). The weights (capital $W$s) can be positive as well as negative, and may have absolute values between zero and infinity. It can be shown that the sum of the weights must be equal to 1, provided the modulation coefficients for the individual complexes (the lower case $w$s) are normalised to the greatest modulation coefficient in the equation. Thus:

For $\varphi_2$: $W_0 + W_\alpha + W_\beta + W_{\alpha\beta} = 1 \quad (9k)$

For $\varphi_3$: $W_0 + W_\alpha + W_\beta + W_\gamma + W_{\alpha\beta} + W_{\alpha\gamma} + W_{\beta\gamma} + W_{\alpha\beta\gamma} = 1 \quad (9l)$
'Nesting'

Thus, in the absence of binding cooperativity between the TFs, the expression for the overall modulation factor $\mu$ can be written as a simple sum. This means that the input type (fractional saturation) is the same as the output type, and that the output of one calculation can be used as input for the next. Thus, when there are three TFs, their contributions to $\mu$ may be split up as follows:

$$\phi = W_0^\mu + W_X^\mu + W_C^\mu + W_{XC}^\mu$$  \hspace{1cm} (10a)

(condition: $W_0^\mu + W_X^\mu + W_C^\mu + W_{XC}^\mu = 1$)

in which:

$$X = W_0^X + W_A^X + W_B^X + W_{AB}^X$$  \hspace{1cm} (10b)

(condition: $W_0^X + W_A^X + W_B^X + W_{AB}^X = 1$)

Substitution of equation 10b in 10a yields equation 9f (with $W_0^\mu = W_0^X \mu$, etc.). Equation 10a is said to be a nested equation when one or more of its terms can be split out into a similar equation.

'Primitives'

Equation 10a is a nested equation when $X$ can be written as another sum of fractional saturation terms, as in equation 10b. However, as long as the types of $X$ and $\phi$ are the same, any expression may substitute $X$, for instance:

$$X = \frac{w_0 + w_A^\alpha + w_B^\beta + w_{AB}^\gamma \alpha \beta}{1 + \alpha + \beta + r_{AB} \alpha \beta}$$  \hspace{1cm} (10c)

(conditions: $0 \leq (w_0, w_A, w_B, w_{AB}) \leq 1$ and $(w_0 \lor w_A \lor w_B \lor w_{AB}) = 1$: the values of all $w$ must be between 0 and 1, and at least one of them must be 1, $\lor$ is logical or). It should be clear that equation 8c can be written as the nested equation 10b, with $W_0^\mu$ and $W_{XC}^\mu$ equal to zero, $X$ as specified in equation 10c, and $C = \gamma/(1 + \gamma)$.

Note, however, that in the last equation the input type (normalised concentration) is not the same as the output type, even though all parameters in the equation are dimensionless. Thus, $\alpha$ and $\beta$ may not be substituted with an expression whose type is fractional saturation.

If $X$ is equal to the right hand side of equation 10c, it cannot be split up any further (unless, of course, $r_{AB} = 1$). It is also impossible to split up the expression for $C$ in equation 10a ($C = \gamma/(1 + \gamma)$). Expressions that cannot be split up any further are called primitives, and all expressions for $\phi$ can be written as a sum of weighted primitives, in which the sum of the weights must be 1. Ligands that bind cooperatively must have their interaction described in a single primitive. The existence of multiple terms in the expression for $\phi$ implies that ligands involved in different primitives bind non-cooperatively.

In addition to primitives such as $X$ in equation 10c, and more complicated forms of the same equation, and simple primitives of the form $A = \alpha/(1+\alpha)$, below are two examples of primitives that have been derived by assuming the binding cooperativity between the ligands is very high.

- **Homo-oligomeric ligands.** Many TFs bind to the cis-regulatory domain as homo-dimers, or higher homo-oligomers. In that case, the fractional saturation of their binding sites are, by approximation, dependent on their normalised concentrations to the power of $h$, where $h$ is a Hill coefficient:

$$A = \frac{\alpha^h}{1 + \alpha^h}$$  \hspace{1cm} (11)

Here, the concentration of $L_A$ has been normalised to the $h^{th}$ root of the dissociation constant for the oligomer to form $\alpha$. Obviously, equation 11 includes the expression for monomers, in which $h = 1$. 

8
**Hetero-oligomeric ligands.** Two or more products from different genes (read: different TFs) may first form a complex, and then bind to the cis-regulatory domain. Strictly speaking, this is a situation in which the ligands bind with high positive cooperativity (e.g. \( r_{ABC} = 1 \), and \( (r_{AB}, r_{AC}, r_{BC}) = r_{ABC} \) in a complex with three TFs). In this case, it is convenient to normalise the concentrations not to the dissociation constant of the individual ligands, but to \( \exp[\Delta G_{\text{complex}}/nRT] \), in which \( \Delta G_{\text{complex}} \) is the free energy difference between the free substrate (no ligands bound), and the fully formed complex with \( n \) species bound: \( a = e^{-\Delta G_{\text{complex}}/nRT} a, \)
\[ \beta = e^{-\Delta G_{\text{complex}}/nRT} b, \text{ etc. For a two-ligand complex, the normalisation factor works out as} \]
\[ (K_a K_b)^{1/2}, \text{ for a three ligand one as } (K_a K_b K_{ABC})^{1/3}, \text{ etc.} \]
The expressions for the fractional saturation of the binding site (or combined binding sites) for hetero-dimers (\( X_2 \)) for hetero-trimers (\( X_3 \)) are:
\[ X_2 = \frac{\alpha \beta}{1 + \alpha \beta} \tag{12a} \]
\[ X_3 = \frac{\alpha \beta \gamma}{1 + \alpha \beta \gamma} \tag{12b} \]
Provided all individual species are present in concentrations at which they do not bind individually (i.e. far below the values of their individual dissociation constants). These expressions can obviously be simply expanded to include more than three ligands. Note that equations 12a and b simplify to equation 11 (with \( h = 2 \) or 3) when \( \alpha, \beta, \text{ and } \gamma \) are products of the same gene.

**Relations between modules**

It is likely that TFs with binding sites on different ‘modules’ within the cis-regulatory domain of a gene will have little effect on each other’s binding behaviour. Thus, the behaviour of a physical module may be described in a single primitive or nested expression in the overall expression for \( \varphi \), the relative activation factor of the gene. In the following, we shall use the term ‘module’ for a single primitive or nested expression in the overall expression for \( \varphi \), and shall leave the ‘sub-’ out.

The relationship between two or more modules is contained in the weights (\( W \), see eqn. 9). The weights can be translated into individual modulation coefficients (w), whose physical meaning (the contributions of particular configurations to the relative activation factor) is easier to understand. The relationships between the modules can be complicated: modules may repress or amplify the effects of other modules. Unfortunately, the resolution of the experimental data on which network models are based is seldom permits such detailed descriptions. However, it is often possible to state, on the basis of available data, that two TFs or TF complexes act together or independently to activate a gene, or that one TF represses the action of another. In those cases, simple logical relations (AND, OR, NOT) often suffice to describe the interaction.

**Gene activation by TF combinations: AND, OR**

If two TFs (or modules), \( L_A \) and \( L_B \), act together to fully activate a gene, whereas the effect of the individual bound TFs is zero, their function may be described as logical AND (\( \land \)). The values for \( w_0, w_A, w_B, \text{ and } w_{AB} \) (equation 9c) in this idealised case are 0, 0, 0, and 1. Equation 9e becomes \( \varphi = A \times B \), since, according to equations 9g-i, \( W_0 = W_A = W_B = 0, \text{ and } W_{AB} = 1 \).

If \( L_A \) and \( L_B \) both activate the gene independently (logical OR, \( \lor \)), the expression for the relative activation factor is \( \varphi = A + B - A \times B \), with \( W_0 = 0, W_A = W_B = 1, \text{ and } W_{AB} = -1 \). \( W_{AB} \) is negative because \( A \) is the set of all configurations in which \( L_A \) is bound to its cis-regulatory binding site. The configuration in which both \( L_A \) and \( L_B \) are bound occurs in both \( A \) and \( B \), and must be subtracted once from their sum.

In fact, the operators \( \land \) and \( \lor \) pertain to operations on Boolean parameters (whose values can be 0 or 1 only), whereas the parameters in equation 9e are continuously valued (0 \( \leq \) \( A \leq 1 \), etc.). Since \( A \) and \( B \)
are sets, the set operators \( \cap \) (cross section) and \( \cup \) (union) are the appropriate operators to describe combined (‘AND’ = cross section, \( A \cap B \)) or independent (‘OR’ = union, \( A \cup B \)) action.

Figure 1a and b show the dependence of \( \varphi \) on the TF concentrations for \( A \cup B \), with \( L_A \) and \( L_B \) as monomers (\( h = 1 \), eqn. 11) or (homo-) oligomers (\( h > 1 \)). Note that higher values of \( h \) make \( \varphi \) look more like a Boolean function, in which the jump from a low value (0) to a high value (1) occurs abruptly at the ‘threshold’ concentrations of \( L_A \) and \( L_B \), \( K_A \) and \( K_B \).

**Repression: NOT**

If \( L_B \) represses activation by \( L_A \), the values of \( w_0, w_1, w_2, \) and \( w_{12} \) are 0, 1, 0, and 0: when \( L_B \) is bound, there is no transcription initiation. The expression for “\( L_A \ AND \ NOT \ L_B \)” (\( L_A \land \neg L_B \)) is: \( A \times (1 - B) \).

In general, if \( L_X \) is a repressor, the factor \( (1 - X) \) will appear as a factor in the equation. Figures 1c and d show a plot of the dependence of \( \varphi \) on \( L_A \) and \( L_B \) in the continuous valued form of \( L_A \land \neg L_B \) for \( L_A \) and \( L_B \) monomers and oligomers.

The kind of inhibition, where \( L_B \) prevents the action of \( L_A \) by binding to an independent binding site, is sometimes called pure non-competitive inhibition. The other forms of inhibition (competitive, uncompetitive, and general non-competitive inhibition), in which there is a certain degree of dependence in the binding of activator and repressor, cannot be expressed so easily or precisely in the Sigma-Pi formalism.

**Combinatorial logic**

Table I summarises the most important unary and binary relationships in cis-regulatory logic. The bold typeface of \( w \) and \( W \) indicates that the weights in equations 3 and 4 are \( 2^N \)-dimensional vectors.

The output of each of these logical and set operations can be used as input to another one, and the rules of Boolean and continuous-valued logic hold (associative and commutative laws, De Morgan’s theorem, etc.). As stated above, in a sequence of operations the input and output types of the functions should be the same. Since the output is fractional saturation, the input to the next function should also be fractional saturation, and expression 4, rather than 3, must be used to evaluate the function value.
Figure 1. Dependence of $\phi$ on the concentrations of two monomeric ($a, b$) or oligomeric ($c, d; h = 4$, see text) TFs that bind independently to their cognate cis-regulatory DNA. $a, c$: bound TF1 or TF2 or both activate transcription ($output = X_1 + X_2 - X_1X_2$); $b, d$: repression by TF2: TF1 promotes transcription, but TF2 inhibits it ($output = X_1(1-X_2)$).
Table I

<table>
<thead>
<tr>
<th>Logical operation</th>
<th>Set operation</th>
<th>Algebraic representations</th>
<th>( w ) (eqn 3)</th>
<th>( W ) (eqn 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>( X_1 )</td>
<td>[ \frac{1}{1 + \xi_1} ]</td>
<td>( 1 - X_1 )</td>
<td>{1, 0}</td>
</tr>
<tr>
<td>TF1 ( \land ) TF2</td>
<td>( X_1 \cap X_2 )</td>
<td>[ \frac{\xi_1 \xi_2}{1 + \xi_1 + \xi_2 + \xi_1 \xi_2} ]</td>
<td>( X_1 \times X_2 )</td>
<td>{0, 0, 1}</td>
</tr>
<tr>
<td>TF1 ( \lor ) TF2</td>
<td>( X_1 \cup X_2 )</td>
<td>[ \frac{\xi_1 + \xi_2 + \xi_1 \xi_2}{1 + \xi_1 + \xi_2 + \xi_1 \xi_2} ]</td>
<td>( X_1 \times (1 - X_2) + X_2 )</td>
<td>{0, 1, 1}</td>
</tr>
</tbody>
</table>

Example: the sea urchin *Endo16* gene

The regulatory interactions that result in activation of the transcription of the sea urchin (*Strongylocentrus purpuratus*) embryo *Endo16* gene have been thoroughly characterised. A comprehensive model of the relationships between 15 cis-regulatory elements, described in a series of IF-THEN-ELSE statements, has been presented previously (Yuh et al. 2001). Figure 2 shows a simplified version of this model, expressed in the Sigma-Pi formalism. In the following, the interactions involving CB2, R, F, E, DG, and Z have been omitted from the model, and the interactions between CY and CB1, CG1 and P, and CG2, CG3, and CG4 have been lumped together. Altogether, the simplified model contains 5 interaction sites: CY/CB1, UI, CG1/P, OTX, and CG2..4 (see figure). It is assumed that the UI and OTX sites are sometimes partially occupied, and that their saturation profile is continuously valued. Boolean values suffice to describe binding thermodynamics of the other TFs.

The *Endo16* cis-regulatory region consists of two physical ‘modules’, A and B. In the early stages in the development of the sea urchin embryo, activation of transcription is determined through interactions on the A module, but in a later stage, transcription control switches to the B module. As stated above, the regulatory region can be modelled as a single Sigma-Pi unit. However, by splitting up the system in multiple Sigma-Pi units that combine to produce the final expression level, the modularity of the system is captured, and individual interactions and mechanisms are made explicit.

Many of the interactions between individual TFs or TF combinations can be described as simple logic gates, but sometimes the weights in the Sigma-Pi units are different from those in the basic logic gates, and must be specified. In particular, occupation of the CY/CB1, CG1/P or CG2..4 sites ‘boost’ the expression level, and this type of synergism between the ‘drivers’ UI and OTX (see (Yuh et al. 2001)) and the other interaction sites cannot be expressed in simple logic terms. Thus, occupation of the CY/CB1 and CG1/P sites amplify the output determined by UI by a factor of 4. The presence of CY/CB1 is not essential, but occupation of the CG1/P site is, and this can be read from the weight matrix for unit \( \Sigma \Pi 1 \). Occupation of the CG2..4 binding sites boost the overall output by an additional factor of 2. All weight matrices are given in Table II, both as \( w \), which can be intuitively understood, and \( W \), whose values are required in the evaluation of Sigma-Pi unit sequences.

Furthermore, the modularised model elucidates the mechanism of the early/late switch in *Endo16* expression, and the central role of CG1 and P. If the CG1/P binding sites are unoccupied, OTX can
activate transcription, whereas UI is inactive. However, if the CG1/P sites are occupied, filling of the UI site results in gradual repression of the action of OTX, so that the expression level is wholly determined by UI once its binding site is fully saturated.

Figure 2. Simplified version of the model for transcription activation of the *S. purpuratus* embryo *Endo16* gene (Yuh et al. 2001). The long (broken) horizontal line signifies the *cis*-regulatory DNA of the gene. The open box to the right indicates the start of the *Endo16* open reading frame, and the boxes on to of the line are TF interaction sites. Interactions between occupied TF binding sites are symbolised by named circles, and arrows indicate the input to the Sigma-Pi functions that are represented by these circles. The bar connection between and2 and OTX indicates repression. The weight matrices for the Sigma-Pi functions are given in Table II.
Table II

<table>
<thead>
<tr>
<th>Unit</th>
<th>Input</th>
<th>Input weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name</td>
<td>#</td>
</tr>
<tr>
<td>ΣΠ</td>
<td>CY/CB1</td>
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</tr>
<tr>
<td></td>
<td>UI</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CG1/P</td>
<td>3</td>
</tr>
<tr>
<td>and</td>
<td>UI</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CG1/P</td>
<td>2</td>
</tr>
<tr>
<td>and</td>
<td>OTX</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Unit 2</td>
<td>2</td>
</tr>
<tr>
<td>or</td>
<td>Unit 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Unit 3</td>
<td>2</td>
</tr>
<tr>
<td>ΣΠ</td>
<td>CG2..4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Unit 4</td>
<td>2</td>
</tr>
</tbody>
</table>

Discussion

We have shown that the simple, but powerful Sigma-Pi formulation can be used to describe activation of gene expression when the following assumptions are made: 1) TF association and dissociation are fast with respect to gene transcription and mRNA translation, and 2) binding of 'primitives' is uncooperative. Here, a primitive is an expression for fractional saturation of a single TF, or a combination of cooperatively binding TFs, in an equation that cannot be simplified further (e.g. eqn 10).

It is likely that there is a significant amount of cooperation in the binding of different TFs to the same cis-regulatory region of a gene. Thus, a precise description of TF binding to a cis-regulatory region would potentially require definition of one or few rather complex primitives. However, the Sigma-Pi formulation can, to a reasonable extent, mimic cooperative behaviour. Suppose individual TFs activate gene expression to a certain degree, but their combination has a much larger effect than would be expected on the basis of their individual contributions. As pointed out above, the cause of the synergism could be cooperative binding (effects on r, e.g. in eqns 5 and 7) or, e.g., enhanced RNAp binding to the combination (effects on w). However, unless detailed data on TF and RNAp binding thermodynamics are available, it will in practice not be possible to distinguish between these possibilities. It can be shown that Sigma-Pi functions with appropriately adjusted weights, using simple primitives (e.g. eqns 3, 11, 12), may be used to approximate more complex binding functions (e.g. eqns 5, 7).

The advantage of describing a GRN as a Sigma-Pi network is that the number of parameters that must be provided or optimised is about half of the number of parameters more complete, but experimentally inaccessible, models. In spite of the fact that they are linear, Sigma-Pi units can, in principle, be used to approximate any function – although in more complex cases the number of required parameters may
proliferate. In the fields of artificial neural networks and evolutionary computation, techniques have been developed to optimise the weights (“train”) in Sigma-Pi networks (Karayiannis and Venetsanopoulos 1995), and infer their structure on the basis of their input-output relation (e.g. genetic programming, see (Koza et al. 1999)). Once a GRN is expressed in Sigma-Pi terms, these techniques may be applied for parameter optimisation and gene network inference.

Furthermore, as we have shown in the example, it may be possible to split a single Sigma-Pi unit that specifies all interactions on a complex cis-regulatory domain into smaller modules. This has several advantages. Firstly, modularisation reduces the total number of weights that must be defined for combinations of interactions (cross products). For instance, it is not necessary to specify a weight for the cross product of the interactions on CY/CB1 and CG2.4 in the model in Figure 2. Obviously, modularisation is justified only when experiments have shown that there is a strong correlation between the effects of particular individual TFs. Secondly, interactions between individual bound TFs are made explicit, and can be visualised and understood more easily than a large Sigma-Pi weights matrix. Thirdly, it is likely that a substantial fraction of all interactions within in a regulatory unit can be approximated by simple logical functions (which are special cases of Sigma-Pi functions). This, again, facilitates intuitive understanding, which may be crucial in determining the course of further experimental work.

### Appendix 1

**Generalised expressions**

Equation A1 gives the generalised expression for the fractional saturation of the \( j \)th combination of ligands on a substrate (cf eqn 7):

\[
y_j = \frac{r_j \prod_{i \in I_j} \xi_i}{\sum_{k=1}^{N} r_k \prod_{i \in I_k} \xi_i}
\]  

(A1)

Here, \( N \) is the total number of ligands, \( \xi_i \) the normalised concentration of the \( i \)th ligand \( L_i \) in the complex, and \( i \in I_j \), \( i \in I_k \), and \( j \in I_k \) indicate inclusion of all \( i \) in the index sets \( I_j \) and \( I_k \) when forming the product of the normalised concentrations \( \xi_i \). Note that the first term in the denominator (for \( k = 1 \)) equals 1, and that \( r_k = 1 \) for \( 1 < k \leq N \).

The generalised expression for transcription activation, relative to a maximum initiation rate (cf eqn 8) is:

\[
\varphi = \frac{\sum_{j=1}^{2^N} w_j r_j \prod_{i \in I_j} \xi_i}{\sum_{k=1}^{2^N} r_k \prod_{i \in I_k} \xi_i}
\]  

(A2)

The meaning of the symbols is the same as in equation A1, and \( w_j \) \((0 \leq w_j \leq 1)\) is the weight of the \( j \)th component in the summation.

Equation A2 simplifies to equation A3 upon assumption that all values of \( r \) are 1.

\[
\varphi = \sum_{j=1}^{2^N} W_j \prod_{i \in I_j} X_i
\]  

(A3)

\( W_j \) are the weights of the components \( j \), and relate linearly to \( w_j \) in equation A2 (see eqns 15 in text). \( X_i \) relates to \( \xi_i \) as follows:
\[ X_i = \left( \frac{\prod_{l=1}^{L_{\text{tot}}} \xi_l^{n_l}}{1 + \prod_{l=1}^{L_{\text{tot}}} \xi_l^{n_l}} \right)_i \]  

(A4)

Here, all ligands that bind in a strongly cooperative way are interpreted as forming a complex \( L \) that binds as a single entity to a binding site on the substrate. The index \( l \) enumerates over all \( L_{\text{tot}} \) species in \( L \), and \( n_l \) is the number of identical molecules of species \( l \) (i.e. the products of a single gene) in \( L \).

References
