Multiplexing information flow through dynamic signalling systems

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Abstract

We provide experimental evidence suggesting that the NF-κB transcription factor can multiplex information about changes in multiple signals in the sense that the NF-κB target genes response can identify which of these signals have changed. In view of this, we consider how a signalling system can act as an information hub by multiplexing multiple signals. We formally define multiplexing, mathematically characterise which systems can multiplex and how well they can do it. We believe this may resolve the apparent paradox of how a system like NF-κB that regulates cell fate and inflammatory signalling in response to diverse stimuli can appear to have the low information carrying capacity suggested by recent studies on scalar signals. In carrying out our study, we introduce new methods for the analysis of large, nonlinear stochastic dynamic models, and develop computational algorithms that facilitate the calculation of fundamental constructs of information theory such as Kullback–Leibler divergences and sensitivity matrices, and link these methods to new theory about multiplexing information. We show that many current models such as those of the NF-κB system cannot multiplex effectively and provide models that overcome this limitation using post-transcriptional modifications.

Keywords: signalling | NF-κB | cellular decision-making | sensitivity analysis | stochastic modelling | system size expansion | oscillations

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1 Introduction

Signalling systems provide a very important example of cellular information systems since they transmit information arising from inside and outside the cell to the cell's processing units. For example, it is generally believed that the NF-κB system uses the information from a large number of input signals (see Fig. 1(a)) to regulate gene transcription of more than 500 genes in a highly versatile way [8, 10]. NF-κB regulates cell fate and inflammatory signalling in response to diverse stimuli, including changes in temperature [9], viral and bacterial pathogens, free radicals, cytokines, and growth factors [10]. Thus, we have a situation where both the input signal $\boldsymbol{S}$ that encodes information about the cell's environment, and the gene response $\boldsymbol{R}$ are multi-dimensional. This raises the question of how a signalling system, where the key signalling molecule is a single transcription factor (TF), can regulate a relationship between multidimensional inputs and responses that can in turn robustly and reliably modulate decision-making of the claimed versatility.

An obvious first question is whether there is experimental evidence that the signalling system is in fact transmitting useful multi-dimensional information. To answer this, we study the response of NF-κB target genes to changes in the physiological signal. These signal changes can be, for instance, changes in temperature or other physical parameters (e.g. pressure or humidity), changes in the level and/or timing pattern of an activator (e.g. tumor necrosis factor-α (TNFα), interleukin 1β (IL-1β), and Lipopolysaccharides (LPS) for NF-κB), and drug treatments (e.g. Diclofenac for NF-κB). We will say that a signalling system can multiplex the input signals $S_1, \ldots S_d$ if one can reliably determine which of these input signals have changed based on the multidimensional response $\boldsymbol{R}$ of the target genes (see Fig. 1(b)). We present experimental evidence below that NF-κB target genes can multiplex a significant number of input signals.

We therefore need to address the question of what aspects of the system enable this multiplexing. To do this we will introduce a quantity, called *multiplexing capacity*, that measures the ability of a noisy signalling system to multiplex a set of signals. The computation of this is underpinned by the pcLNA method [17] that allows fast and accurate computation of key information theoretic quantities, such as Kullback-Leibler divergences and the Fisher Information matrix [5], for stochastic dynamical systems.

Given that NF-κB has complex oscillatory dynamics, an obvious hypothesis is that it is this dynamical behavior of the system that allows the extra information that is transmitted via multiplexing. However, we will provide evidence that this is not necessarily the case and show that the NF-κB system described by current models cannot multiplex effectively even though it has oscillatory dynamics. On the other hand, we will demonstrate how to modify a stochastic model of NF-κB so as to overcome this inability to multiplex. In particular, we show that additional regulated states of NF-κB, which might include differential post-translational modifications and/or differential hetero- and homo-dimerisation, can enable such multiplexing and that the oscillatory dynamics can greatly enrich the multiplexing capacity in this modified model.

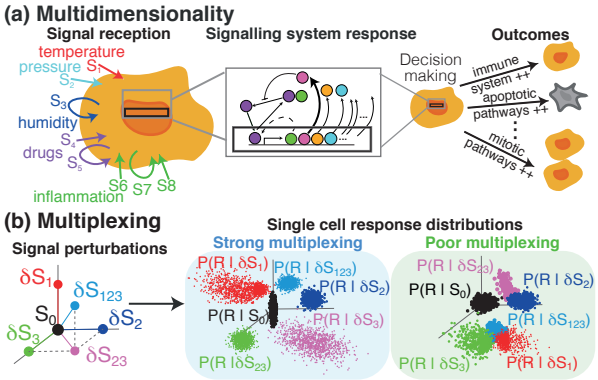

Figure 1: **Multiplexing signals through signalling systems** (A) Cells constantly receive a multitude of different signals in which signalling systems respond by (directly or indirectly) modulating the expression of a number of target genes. These target genes activate or not various pathways of the cell which leads to completely different cell outcomes from cell survival to apoptosis or mitosis. In order for this decision making to be done reliable and robustly, signalling systems need to have the capacity to multiplex a variety of simultaneously arising signals. (B) Multiplexing is defined as the ability of a signalling system response to identify which of the signals have changed. In broad terms, strong multiplexing is evident by the probability distributions of the signalling system response in a population of single cells being significantly different for the different regimes of the multi-dimensional signal. On contrary, poor multiplexing leads to response distribution that are very similar for different signals.

Recent important papers studied the information flow through biochemical systems such as the nuclear factor-κB (NF-κB), calcium (Ca^{2+}), and extracellular signal-regulated kinase (ERK). The focus has been on measuring how much information is being carried by the signalling systems in terms of the mutual information $I(S, R)$ and the capacity of the channel $S \to P(R|S)$ [5] where S is the input signal and $P(R \mid S)$ the probability distribution of possible responses R. In summary, the channel capacity was estimated to be around 1 bit for static scalar observations in response to one-dimensional stimuli [2, 4, 20, 25, 30], about 1.5 bits when the dynamical behaviour of the system response is considered [25] and up to 1.7 bits when cell-to-cell heterogeneity is accounted for [33]. A number of recent studies support this core observation and report similar low channel capacitites [12, 13, 15, 16, 29, 43]. We also show that this level of channel capacity is consistent with the stochastic models of the systems that we study here and that of the stochastic model in [27].

The evidence we supply that NF-κB can multiplex, it also suggests that when the signal **S** is defined as in Fig. 2 and **R** is the response of the NF-κB target genes listed there, the channel capacity is several bits. This raise the question of how our results relate to the significantly lower channel capacity reported in the above papers. We will address this question more carefully below. However, note that these results are not contradictory because, for the

modified NF-κB model which has high multiplexing capacity, the signal $\boldsymbol{S}$, the transcription factor $\mathbf{T}$, and the target genes $\mathbf{G} = \mathbf{R}$ are all multidimensional. In particular, the transcription factor $\mathbf{T}$ has two gene regulatory states which allows for much bigger amounts of information to be transferred.

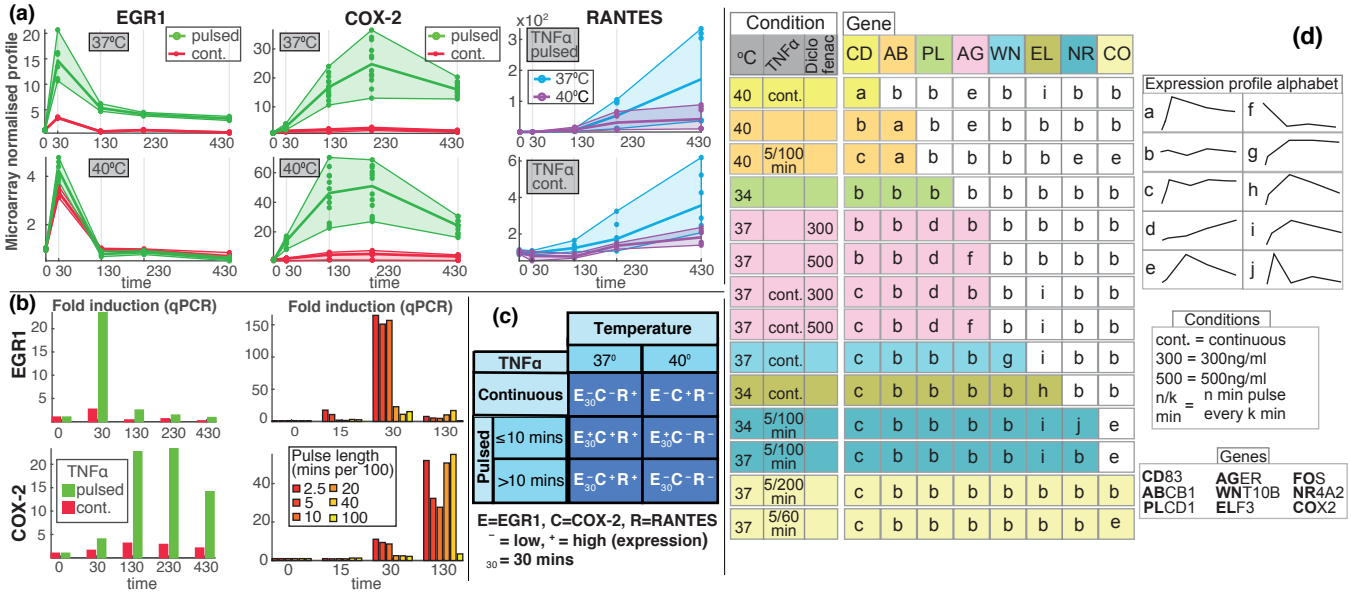

Figure 2: **Gene expression can identify different experimental conditions.** (A) The expression of three genes EGR1, COX-2 (PTGS2) and RANTES (CCL5) as measured by microarrays in normal (37°C) and high (40°C) temperature and under continuous or pulsed (5min stimulation every 100mins, see legends) TNFα treatment. EGR1 and COX-2 are differentially expressed between the two different TNFα treatments, while RANTES between the two temperatures. (B) RT-qPCR for EGR1 (up) and COX-2 (below) for pulsed (left: 5/100mins, right: see legend) and continuous TNFα treatment in normal temperature. (C) Summary table of the 3 genes' expression profiles that can identify 6 different conditions. (D) Summary table of the expression of 8 genes (columns) that can distinguish the 14 different experimental conditions (rows). Each letter corresponds to a different normalised expression profile given on the right. Reading the table from left to right and top to bottom, the colours emphasise the gene that identifies each condition. The profile data are in SI Fig. 2.

Does NF-κB multiplex?

To illustrate the above characterisation of multiplexing we consider some experimental evidence that NF-κB can multiplex. We ask if, by monitoring the response of a set of genes that are direct NF-κB targets (SI Sect. 5), we can reliably determine the state of a multidimensional input signal. We firstly consider the response of three important genes, EGR1, COX-2 and

RANTES, to pulses of varying length repeated every 100 minutes at two temperatures, 37°C and 40°C and ask if, from the response of these genes, we can determine the temperature and pulsing length. EGR1 regulates the response to growth factors, DNA damage, and ischemia, preventing tumor formation by activating p53/TP53 and TGFB1. COX-2 (PTGS2) is responsible for production of inflammatory prosta-glandins. RANTES (CCL5) is a chemotactic cytokine for T cells, eosinophils, and basophils. It plays an active role in recruiting leukocytes and the proliferation and activation of certain natural-killer cells.

We use microarrays and RT–qPCR data to monitor the expression of these genes around the peak times of nuclear NF-κB at 0, 30, 130, 230 and 430 minutes (Fig. 1(a-b)). We see that, if we know the expression levels of these genes at these times we can determine which of these multiple experiments was carried out (Fig. 1 (c)). Since there are 6 distinct states in the table we see that just from monitoring these three genes we obtain more than 2 bits of information.

That significantly more information is actually being transmitted is shown in Fig. 1(d). To each of the 14 considered experimental conditions and each of the 8 genes we attach one of 10 expression profiles labelled a-j. We see that if we know the expression profiles of these genes we can determine which of the 14 experimental conditions was used. This appears to uncover just under 4 bits of information.

These results are not definitive since we are using data from cell population assays such as microarrays and consequently this is a result about cell populations rather than single cells. Moreover, we have neglected stochastic effects that will be very important when single cells are considered. Nevertheless, these results are highly suggestive and motivate a careful consideration of single cell multiplexing.

TF multi-dimensionality

If one assumes that that the level T of the NF-κB transcription factor in the nucleus is what determines the target gene expression $G = R$ (i.e. $S \to T \to G$ is a Markov chain) then by the data processing inequality [5] the channel capacity of the chain $S \to G$ must be at most equal to that of both $S \to T$ and $T \to G$ and thus in the range 1-1.7 bits. On the other hand, it is worth pointing out that if this is the case and if $\mathbf{T} = (T_1, T_2)$ where T_1 is the total amount of nuclear transcription factor and T_2 is the amount in a modified form, then the channel capacity of $S \to \mathbf{T} = (T_1, T_2)$ can be arbitrarily larger than the measured channel capacity $S \to T_1$ even when S is scalar.

The combination of the low channel capacity of [25] and the data processing inequality implies that if one accepts that the true channel capacity is several bits, as suggested by the experimental results above, then T must be multidimensional in the sense that it cannot be a function of the TF level alone. This motivates our use of additional gene regulatory states.

Results

Decision-making and KL divergence

We now develop a mathematical theory that enables us to quantify multiplexing. We use this to show why current tightly coupled models of NF-κB cannot multiplex effectively and then explain how to modify these so that multiplexing is enabled.

Suppose we have s signals $S_1, \ldots, S_s$ which in turn define the vector signal $\boldsymbol{S} = (S_1, \ldots, S_s)$. Consider a change in the signal from a base value $\boldsymbol{S}_0$ to $\boldsymbol{S} = \boldsymbol{S}_0 + \boldsymbol{\delta S}$ where the change has size $\eta = ||\boldsymbol{\delta S}||$. We ask whether, the response $\boldsymbol{R}$ has the capacity to distinguish which components of the signal have significantly changed i.e. which components of $\boldsymbol{\delta S}$ are $\geq \mathrm{O}(\eta)$.

Mathematically the question of using the stochastic response $\boldsymbol{R}$, which has probability distribution $P_{\boldsymbol{S}}(\boldsymbol{R}) = P(\boldsymbol{R}|\boldsymbol{S})$, to distinguish input signals is related to hypothesis testing. If we wish to determine whether, in moving the signal from $\boldsymbol{S}_0$ to $\boldsymbol{S} = \boldsymbol{S}_0 + \boldsymbol{\delta S}$, the ith component has changed, we need to be able to evaluate the hypothesis that $\boldsymbol{R}$ comes from $P_{\boldsymbol{S}}$ rather than from a distribution of the form $P_{\boldsymbol{S}'}$ where $\boldsymbol{S}'$ is any perturbation of $\boldsymbol{S}_0$ with the same ith component as $\boldsymbol{S}_0$. By the Neyman-Pearson lemma, the most powerful test of this hypopthesis for a given false-positive error rate α is a test of the form $\lambda(\boldsymbol{R}) \geq u_\alpha$ where

$$\lambda(\boldsymbol{R}) = \log \frac{P_{\boldsymbol{S}}(\boldsymbol{R})}{P_{\boldsymbol{S}'}(\boldsymbol{R})}$$

is the log-likelihood ratio and the choice of α determines what threshold u_α to use. The $P_{\boldsymbol{S}}$-mean of the log-likelihood ratio is by definition the Kullback–Leibler (KL) divergence, $D_{\mathrm{KL}}(P_{\boldsymbol{S}}||P_{\boldsymbol{S}'})$, of $P_{\boldsymbol{S}}$ and $P_{\boldsymbol{S}'}$ distributions. The larger is the likelihood ratio, the more evidence we have in favor of signal $\boldsymbol{S}$ and against $\boldsymbol{S}'$.

If D_{KL} is too small then the most powerful test is expected to fail and hence other tests will not fair any better. Furthermore, as we wish to check whether the response $\boldsymbol{R}$ has the capacity to distinguish $\boldsymbol{S}$ from *any* $\boldsymbol{S}'$ that has the i-th signal unchanged, we study how large is the $\min_{\boldsymbol{S}' \in \mathcal{S}_{(i,0)}} D_{\mathrm{KL}}(P_{\boldsymbol{S}}||P_{\boldsymbol{S}'})$ where $\mathcal{S}_{(i,0)}$ is the set of all such $\boldsymbol{S}'$ signals. However, as $\boldsymbol{S}$ tends towards $\boldsymbol{S}_0$ thus decreasing the length $l = l(\boldsymbol{S}) = \|\boldsymbol{S} - \boldsymbol{S}_0\|$, this quantity decreases like l^2, and therefore we scale it and define

$$D_{\mathrm{KL}}^{(i,\boldsymbol{S}_0)} = \min_{\boldsymbol{S}} l(\boldsymbol{S})^{-2} \min_{\boldsymbol{S}' \in \mathcal{S}_{(i,0)}} D_{\mathrm{KL}}(P_{\boldsymbol{S}}||P_{\boldsymbol{S}'}). \tag{1}$$

The larger $D_{\mathrm{KL}}^{(i,\boldsymbol{S}_0)}$ is, the easier it is to detect the change in the ith component.

To apply this so as to detect changes in any component of the signal $\boldsymbol{S}$ we consider

$$\mathrm{MX}(S_1, \ldots S_s) = \min_{i=1,\ldots,s} D_{\mathrm{KL}}^{(i,\boldsymbol{S}_0)}. \tag{2}$$

The larger this *multiplexing capacity* $\mathrm{MX}(S_1, \ldots S_s)$ is, the better the system at multiplexing the signals $S_1, \ldots S_s$.

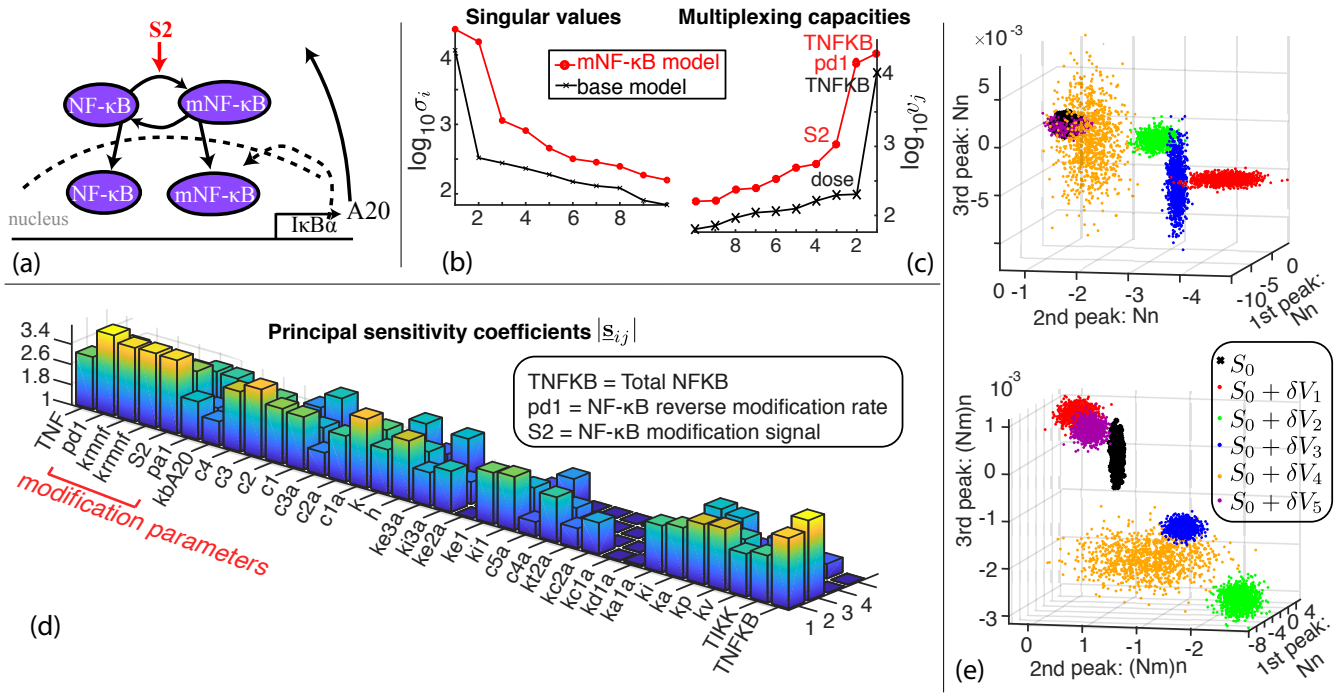

Figure 3: **Comparisons of the multiplexing capacities and sensitivities of the base NF-κB and mNF-κB models.** (a) Diagram of the reactions leading to the reversible modification of cytoplasmic NF-κB in the mNF-κB model. (b) The singular values σ_i of the base model and the much larger σ_i values of the mNF-κB model. (c) The multiplexing capacities v_j of the base and mNF-κB models. The parameters with the largest multiplexing capacities correspond to TNFKB and TNF dose for the base model and TNFKB, pd1 and S_2 (modification parameters) for the mNF-κB model. (d) The principal sensitivity coefficients of the mNF-κB model. Larger values indicate higher sensitivity of the mNF-κB model to changes in the value of the corresponding parameter. (e) Realisations (n=1000) of the pcLNA distributions of the base (top) and mNF-κB model (bottom) at three phases (see x-,y-,z-axis) of the NF-κB dynamics, for the standard parameter values, $\boldsymbol{S}_0$, and with the parameter values changed by $\delta = 10\%$ in the FIM eigen-directions $V_1, \ldots, V_5$ corresponding to the 5 largest eigenvalues of each model. The result illustrates the much larger sensitivity of the probability distribution of the mNF-κB model compared to the base NF-κB model to changes in the parameter values.

Characterising multiplexing via the sensitivity matrix

While we cannot calculate this quantity tn general, we can find an elegant solution in terms of the Fisher Information Matrix (FIM) when the changes in the signal are small, that is the third order terms and above are negligible. That is, we will calculate $D_{\mathrm{KL}}(P_{\boldsymbol{S}} \| P_{\boldsymbol{S}'})$ up to terms that are $\mathrm{O}(\max\{\|\boldsymbol{S} - \boldsymbol{S}'\|^3, \|\boldsymbol{S} - \boldsymbol{S}_0\|^3, \|\boldsymbol{S}' - \boldsymbol{S}_0\|^3, \})$.

In our context, the FIM $\mathcal{I}$ at $\boldsymbol{S}_0$ has entries

$$\mathcal{I}_{ij} = \mathbb{E}_{P_{\boldsymbol{S}_0}} \left(\partial_i \ell \cdot \partial_j \ell\right) = -\mathbb{E}_{P_{\boldsymbol{S}_0}} \left(\partial^2_{ij} \ell\right)$$

where $\ell(\boldsymbol{S}\,; R) = \log P(\boldsymbol{R}|\boldsymbol{S})$ is the log–likelihood function, $\partial_i \ell$ denotes the partial derivative with respect to the ith component S_i and ∂^2_{ij} is the corresponding second derivative. These derivatives are evaluated at $\boldsymbol{S}_0$.

The FIM measures the sensitivity of $P_{\boldsymbol{S}}(\boldsymbol{R})$ to a change in the signal $\boldsymbol{S}$ because, up to terms that are $\mathrm{O}(\|\boldsymbol{\delta S}\|^3)$ (see SI Sect. 2.1),

$$D_{\mathrm{KL}}(P_{\boldsymbol{S}_0+\boldsymbol{\delta S}}(\boldsymbol{R}) \| P_{\boldsymbol{S}_0}(\boldsymbol{R})) = \boldsymbol{\delta S}^T \, \mathcal{I} \, \boldsymbol{\delta S}/2.$$

One can associate to the FIM $\mathcal{I}$ an $s \times s$ matrix $\underline{\mathbf{s}}$ that satisfies $\mathcal{I} = \underline{\mathbf{s}}^T \underline{\mathbf{s}}$ and certain optimality properties described in [17] (SI Sect. 2.5.2). We call the entries, $\underline{s}_{ij}$, of matrix $\underline{\mathbf{s}}$, *principal coefficients of sensitivity* of the response $\boldsymbol{R}$ to the j-th signal S_j, $j = 1, 2, \ldots, s$. The sensitivity matrix $\underline{\mathbf{s}}$ describes the ability of the signalling system to multiplex at least locally in the following way.

We denote by $\underline{\mathbf{s}}_j$, j=1, ..., s, the columns of $\underline{\mathbf{s}}$, by $\underline{\mathbf{s}}_{i_1,\ldots,i_k}$ the linear subspace of $\mathbb{R}^s$ spanned by the vectors $\underline{\mathbf{s}}_{i_1}, \ldots, \underline{\mathbf{s}}_{i_k}$ and by $\mathbf{n}$=$\mathbf{n}(i|i_1, \ldots, i_k)$ the component of $\underline{\mathbf{s}}_i$ normal to the linear subspace $\underline{\mathbf{s}}_{i_1,\ldots,i_k}$ i.e. $\underline{\mathbf{s}}_i = \mathbf{u}+\mathbf{n}$ with $\mathbf{u}$ in $\underline{\mathbf{s}}_{i_1,\ldots,i_k}$ and $\mathbf{n}$ orthogonal to $\underline{\mathbf{s}}_{i_1,\ldots,i_k}$. If $i_1, \ldots, i_k$ include all indices except j we use the notation $\mathbf{n}(i|j \neq i)$.

Firstly, up to third order terms,

$$D_{\mathrm{KL}}^{(i,\boldsymbol{S}_0)} = \|\mathbf{n}(i|j \neq i)\|^2/2,$$

and therefore the length of the normal component, $\mathbf{n}(i|j \neq i)$, determines, at least locally, the capacity of the response $\boldsymbol{R}$ to distinguish the i-th from the rest of the considered signals.

Secondly, there is an essentially unique reordering of the signal components as $S_{i_1}, \ldots, S_{i_s}$ so that if $v_k = ||\mathbf{n}(i_k|i_1, \ldots, i_{k-1})||$ then $v_1 \geq \cdots \geq v_s$ and the multiplexing capacities

$$\mathrm{MX}(S_{i_1}, \ldots S_{i_k}) = v_k^2/2. \tag{3}$$

All of these quantities can be rapidly calculated using the QR decompositions of submatrices of $\underline{\mathbf{s}}$ made up from the relevant columns of $\underline{\mathbf{s}}$.

This ordering of the set of signals provides a way to choose an optimal subset that can multiplex. That is, we can use the ordering $i_1, \ldots, i_s$ and the associated multiplexing capacities $\mathrm{MX}(S_{i_1}, \ldots, S_{i_k})$, $k = 1, \ldots, s$, to identify the subset of signals with the largest number of elements, k, that has multiplexing capacity $\mathrm{MX}(S_{i_1}, \ldots, S_{i_k}) \geq m$, for m an appropriate threshold (e.g. the minimum D_{KL} level for the change to be detectable in a given system of interest).

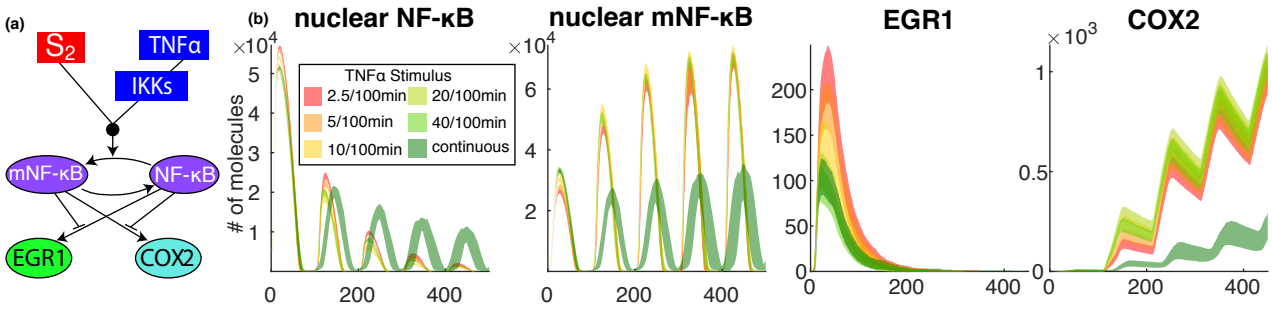

Figure 4: **Stochastic simulation of a model of mNF-κB allowing to describe the regulation of EGR1 and COX-2 genes for the same types of TNFα stimulation as in Fig. 1.** (a) Diagram of the network and (b) 95% confidence envelopes of the number of nuclear NF-κB and mNF-κB , and EGR1 and COX-2 mRNA copies under continuous TNFα treatment and treatment with pulses of TNFα stimuli of various durations derived using stochastic simulations.

Tightly coupled models of NF-κB cannot multiplex effectively

One might expect that a dynamical system with many parameters would have the flexibility to multiplex effectively. However, it has been observed that for a large class of deterministic models of regulatory and signalling systems of the sort that we are considering, the deterministic analogue of the FIM for the model parameters has rapidly decreasing eigenvalues σ_i^2 [7, 22, 23, 24, 28, 31]. A similar result was shown for stochastic models of the circadian clock in [17]. This implies that the effects of changing different parameters are highly correlated making it hard to recognise which parameter was changed.

If the signals S_i act through changing parameters θ_j of the system (i.e. $\theta = \theta(\boldsymbol{S})$) then a similar result is true for the signal FIM $\mathcal{I}$ (SI Eq. (1)). We see in Fig. 2(b) that such a rapid decline is the case for the base model considered here. The singular values of the FIM decay with an exponential rate. The second singular value is less than 1% of the first one.

But $\mathcal{I} = \underline{\mathbf{s}}^T \underline{\mathbf{s}}$ and therefore the eigenvalues of the FIM $\mathcal{I}$ are the squares of the singular values σ_i of $\underline{\mathbf{s}}$ (SI Sect. 2.3). The singular values σ_i rapidly decrease and, since $v_1 \cdots v_k \leq \sigma_1 \cdots \sigma_k$ for all $k \leq s$ with equality when $k = s$ (Theorem 3.3.2 of [11], see SI section 1) the same is true for the multiplexing capacities v_j, $j = 1, \ldots, k$. Using equation (3) we see that the set of signals that can multiplex well must be very small. Fig. 2(c) shows how fast the v_j decrease for our base model and identifies the parameters through which signal can multiplex more effectively i.e. these are the parameters that the signals should move if the signals are to be effective.

Intuitively, the reason behind these results is an *orthogonality principle*: in order to distinguish a change δS_i of the signal in the ith direction from one δS_j in the jth one, these changes must move $P_{\boldsymbol{S}}(\boldsymbol{R})$ in the space of distributions in directions that are close to orthogonal in the sense that $\delta S_i^T \mathcal{I} \delta S_j \approx 0$. If all but k of the eigenvalues of $\mathcal{I}$ are very small then $\mathcal{I}$ is close to

having rank k so such orthogonality can work for at most k signal directions.

Additional regulated NF-κB states

Regulation of the NF-κB pathway is enabled by multiple post-translational modifications that control the activity of the core components of NF-κB signaling. In particular, the RelA NF-κB subunit undergoes reversible modifications such as phosphorylation, ubiquitination, and acetylation that can affect its transcriptional functions [19, 35, 36, 37, 38, 39, 40]. Indeed many modification sites in RelA have been identified as having either an enhancing, inhibitory, or modulatory effect on NF-κB transcriptional activity in a gene-specific manner [19, 35, 36, 37, 39]. A further potentially regulated step that could differentially control individual gene expression is the hetero- and homo-dimerisation of the NF-κB Rel proteins [42, 41]. Therefore, in considering the nature of biological mechanisms that could underlie multiplexing of information by the NF-κB system, it is natural to consider modifications that create additional regulated NF-κB states that can affect the transcription of NF-κB target genes.

We consider one of the simplest modifications of the base model that can enable more effective multiplexing. In this modified model, which we call mNF-κB, the cytoplasmic NF-κB is reversibly modified by an input signal S_2 that is independent to the TNFα signal. The modified form of NF-κB, mNF-κB, competes with the unmodified form for binding of IκBα but otherwise is subject to the same reactions (see SI Sect. 3.3). Importantly, mNF-κB can activate, inhibit, or modulate the transcription of target genes which their differential expression can potentially reveal the levels of S_2 signal.

The mathematical analysis of the stochastic version of the mNF-κB model confirms this. We see that the singular values of the FIM are overall increased, but most importantly, there are now two large singular values rather than one (Fig. 3(b)) and at least two parameters that can multiplex (Fig. 3(c)). The principal sensitivity coefficients, which are large for the parameters related to the modification, confirm that the extra sensitivity arises from the addition of this modification (see Fig. 3(d)). This extra sensitivity is reflected in the probability distributions of the nuclear NF-κB that are much more sensitive to changes in the parameter values than the base model.

Note that the results presented in Fig. 3(b-d) are derived for the probability distributions of stochastic trajectories of the system observed at 9 timepoints. If instead only two time-points are considered, the base NF-κB model is not largely affected, but the mNF-κB presents a clearly less prominent increase of the singular values. This suggests that while the dynamical behaviour of the system does not ensure high multiplexing capacity, it can greatly enhance multiplexing in a system that has the ability to multiplex.

Furthermore, the greater sensitivity of the mNF-κB model compared to the base model is also reflected in Fig. 3(e). We see that the nuclear concentrations of NF-κB are much more affected in the mNF-κB by changes in the signal. Note that these simulations are derived using the pcLNA method described in the section "Stochastic dynamics of NF-κB".

Reproducing multiplexing in the EGR1-COX-2 example

To further illustrate multiplexing, we now consider how to modify the signalling system so as to be able to reproduce the multiplexing behaviour seen in the EGR1-COX-2 example. We are not claiming that this is the true underlying biological mechanism but are using this example to illustrate how the NF-κB signalling system can multiplex different signals through gene regulation. This is clearly not possible under the structural constraints of the base model because: (a) the base NF-κB model under treatment with TNFα pulses acts as a forced oscillator of nearly identical cycles and therefore it cannot explain the difference between early and late expression of EGR1 and COX-2, and (b) the differences in the base model between the response to short and long pulse are extremely small and can hardly explain the differences in EGR1 early response between the different pulse lengths.

The system is modified as shown in Fig. 4(a) to include a reversible modification of NF-κB molecules in the cytoplasm (see also SI section 3). The NF-κB modification is promoted by the TNFα stimulus through the IKK module and the independent signal S_2. Pulses of TNFα cause bursts of NF-κB nuclear translocations, but also higher levels of the modified NF-κB. The reverse modification is independent of S_2 and TNFα. Our model postulates that NF-κB activates the transcription of EGR1, which is inhibited by the mNF-κB, while the reverse regulation is imposed on COX-2. Using our approach to stochastic simulation outlined next, we can calculate the confidence limits for COX-2 and EGR1 under the various pulsing protocols (see Figure 3(b)). The introduction of the additional regulatory states of NF-κB allows us to reproduce the experimentally observed profile.

Stochastic dynamics of NF-κB

The base model used in our analysis is a stochastic reaction network that describes the oscillatory response of the NF-κB system under stimulation by TNFα. It is a slight modification of the system model in [1]. In our version of the model, after adjustments to the rate equations, concentrations are all expressed in terms of the same volume Ω (taken to be Avogadro's number in the appropriate molar units multiplied by the volume of the cell in appropriate units so that Ω has units L/nM (SI Sect. 3.3). The original model is written in terms of nuclear and cytoplasmic concentrations. Clearly, it is straightforward to convert between the two models (see SI Sect. 3.3).

We use the pcLNA stochastic version of this model [17] that allows us to derive analytical expressions for the FIM and system sensitivity matrix $\underline{\mathbf{s}}$ and to rapidly simulate the system with high accuracy (see Figure 4 and SI Section 4.2). The stochastic model considered here converges to the published deterministic model of [1] as $\Omega \to \infty$.

The mNF-κB model that includes NF-κB modification is also simulated and analysed using pcLNA (see SI Section 3.4). For the simulation of downstream genes that are regulated by NF-κB (see next section) we use the Stochastic Simulation algorithm (SSA) [6]. This is because the relevant distribution for the gene expression is far from being Gaussian and therefore it is

not appropriate to apply the pcLNA directly to this subsystem. Since this part of the system involves relatively few molecules the combined system can be simulated rapidly. The SSA is also used for comparisons to pcLNA in Figure 4.

Capacity of scalar channels

The results above raise the question of whether our models are compatible with the channel capacity seen in previous publications. We can use the base model to compare its behaviour with that discussed in [4, 25]. In these papers there was no attempt to control cell size or consideration of total amount of NF-κB (see also [33] which discusses such issues). We therefore allow these quantities to vary with the variation being drawn from a log-Normal distribution as described in SI Sect. 4.4.

We study the case where $\boldsymbol{S}$ is the level of the continuous TNFα stimulation (the parameter *dose*) and the response $\boldsymbol{R}$ is the level of nuclear NF-κB at q different phases including its first peaks and troughs. Fig. 4(d)(i) shows the estimated capacity as a function of q. We also estimate the channel capacity for response R the nuclear concentration at $t = 30$min after initiating continuous TNFα stimulation (Fig. 4(d)(ii)).

The model reproduces the rather limited channel capacity seen in [4, 25] with estimated carrying capacities in the region of one bit. The exact value is not important because this is subject to estimates of Ω, the total concentration of NF-κB molecules and other parameters derived in [1]. A similar result can be derived by the model in [27] (see SI section 4.5).

Discussion

Cells present a very different context from that of traditional communications channels. The genetic and epigenetic information contained in the genome is translated by molecular interactions into dynamical processes. Described by dynamical interaction networks, these stochastic dynamical processes effectively move information from one system to another by regulating the probability distributions of their component molecules. Therefore, it is unclear whether the classical tools are always the most appropriate and it is likely that a much more extensive information toolbox is needed. New ideas about stochasticity and information are needed to understand how cells respond to dynamic environments so as to ensure appropriate cellular responses with high probability when they are using biochemistry that itself is very noisy.

Using such information theoretic tools we suggest a new insight into the way in which signalling systems transmit information. We propose that although they may be rather limited in the way that they transmit any scalar signal they are well designed to transmit multi-dimensional signals. While the amount of information in each dimension can be relatively small the capacity of the multi-dimensional signal may be much larger. As well as partial evidence that NF-κB activation in response to TNFα does this, we provide a mathematical

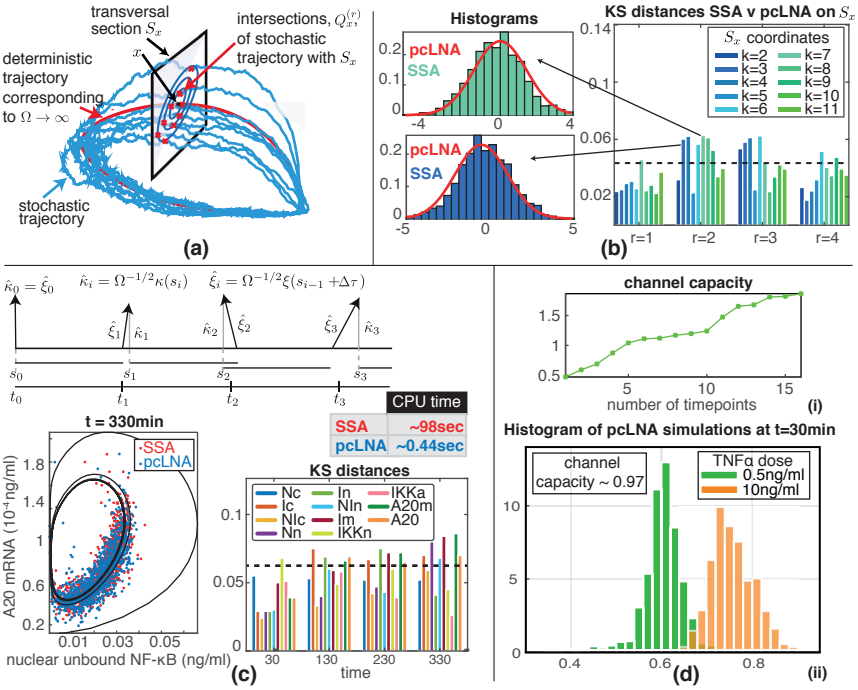

Figure 5: **The pcLNA stochastic model and the channel capacity of the NF-κB model.** (a) The pcLNA model uses the stability of the probability distributions of stochastic oscillatory systems on the transversal sections, S_x, of a given phase, x, of the system's deterministic solution. (b) The pcLNA probability distributions on those transversal sections match very well the empirical distributions derived by SSA. Here the comparison is done using the Kolmogorov-Smirnov (KS) test at the first 4 peaks of NF-κB model. The corresponding histograms for two of the largest observed KS values are also displayed to illustrate the nearly perfect match of the two distributions even in the case of the largest KS distances recorded here. (c) The pcLNA simulations also match very well the SSA simulations of the NF-κB model which is much slower (see CPU (average) time for a single simulation). (d) Estimation of the channel capacity using the pcLNA simulation algorithm with added noise on the Total number of NF-κB molecules.

theory which characterises which signalling systems can perform this multiplexing effectively that also clarifies how to characterise signalling information. This provides a mathematical and conceptual framework for the idea that signalling systems like NF-κB are signalling hubs able to take in multiple inputs and signal to the genes in a way that regulates multiple responses. In particular, it clarifies how a single transcription factor can carry out such a complex task.

Recent research has shown that several important signalling systems such as ERK, NF-κB, N-FAT/Crz1, Stat/Smad/Hes1 and p53 have dynamic oscillatory or cyclic behaviour [1, 3, 14, 18, 21, 26, 32]. This raises intriguing questions about the role of dynamics in information transfer including the suggestion that dynamic systems can transmit greater

amounts of information compared to static/equilibrium systems. Our examples, also suggest why an oscillating system can use multiplexing to transmit more information than equilibrium systems. In these we see that signals that affect protein modification states or other aspects such as dimerization or binding partners can be good for multiplexing. In an equilibrium system the probability distribution describing how these states are distributed will be stationary in time. On the other hand in an oscillatory system these states can have a non-trivial temporal structure (e.g. oscillating) as catalysts of modifications can be activated and deactivated by interaction with the oscillations. This suggests a clear advantage for oscillating systems for information transfer.

Supporting information

S1 Appendix. Details of the mathematical analysis, description of the computational algorithms, and the models used, and additional figures.

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