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Colored extrinsic fluctuations and stochastic gene expression

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Stochasticity is both exploited and controlled by cells. Although the intrinsic stochasticity inherent in biochemistry is relatively well understood, cellular variation, or 'noise', is predominantly generated by interactions of the system of interest with other stochastic systems in the cell or its environment. Such extrinsic fluctuations are nonspecific, affecting many system components, and have a substantial lifetime, comparable to the cell cycle (they are 'colored'). Here, we extend the standard stochastic simulation algorithm to include extrinsic fluctuations. We show that these fluctuations affect mean protein numbers and intrinsic noise, can speed up typical network response times, and can explain trends in high-throughput measurements of variation. If extrinsic fluctuations in two components of the network are correlated, they may combine constructively (amplifying each other) or destructively (attenuating each other). Consequently, we predict that incoherent feedforward loops attenuate stochasticity, while coherent feedforwards amplify it. Our results demonstrate that both the timescales of extrinsic fluctuations and their nonspecificity substantially affect the function and performance of biochemical networks.
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Introduction

Biochemical networks are stochastic: fluctuations in numbers of molecules are generated intrinsically by the dynamics of the network and extrinsically by interactions of the network with other stochastic systems (Elowitz *et al*, 2002; Swain *et al*, 2002). Stochastic effects in protein numbers can drive developmental decisions (Arkin *et al*, 1998; Maamar *et al*, 2007; Nachman *et al*, 2007; Suel *et al*, 2007), be inherited for several generations (Rosenfeld *et al*, 2005; Kaufmann *et al*, 2007), and have perhaps influenced the organization of the genome (Swain, 2004; Becskei *et al*, 2005). Intrinsic fluctuations are generated by intermolecular collisions affecting the timing of individual reactions. Their strength is increased by low copy numbers. The source of extrinsic fluctuations, however, is mostly unknown (Kaern *et al*, 2005), although cell cycle effects (Rosenfeld *et al*, 2005; Volfson *et al*, 2006) and upstream networks (Volfson *et al*, 2006) contribute. Yet extrinsic fluctuations dominate cellular variation in both prokaryotes (Elowitz *et al*, 2002) and eukaryotes (Raser and O'Shea, 2004). They are colored, having a lifetime that is not negligible but comparable to the cell cycle (Rosenfeld *et al*, 2005), and they are nonspecific, potentially affecting equally many molecules in the system (Pedraza and van Oudenaarden, 2005). They are thus difficult to model and their effects hard to predict (Austin *et al*, 2006; Cox *et al*, 2006; Geva-Zatorsky *et al*, 2006; Scott *et al*, 2006; Sigal *et al*, 2006; Tanase-Nicola *et al*, 2006; Tsimring *et al*, 2006; Volfson *et al*, 2006; Maithreye and Sinha, 2007).

Intrinsic and extrinsic stochasticity can be measured by creating a copy of the network of interest in the same cellular environment as the original network (Elowitz *et al*, 2002). We can then define intrinsic and extrinsic variables, and their fluctuations generate intrinsic and extrinsic stochasticity (Swain *et al*, 2002). Intrinsic variables typically specify the copy numbers of the molecular components of the network. Their values differ for each copy of the network. Extrinsic variables often describe molecules that affect equally each copy of the network. Their values are therefore the same for each copy. Considering gene expression, the number of transcribing RNA polymerases is an intrinsic variable (it is different for each copy of the network), whereas the number of cytosolic RNA polymerases is an extrinsic variable (both

copies of the network are exposed to the same cytosolic RNA polymerases).

Stochasticity is quantified by measuring an intrinsic variable, for example, the number of proteins, for both copies of the network. Fluctuations of the intrinsic variable will have intrinsic and extrinsic components: intrinsic variables are themselves part of a stochastic system and that system interacts with other stochastic systems. Throughout we will use the term 'noise' to exclusively mean an empirical measure of stochasticity, usually the coefficient of variation. Experimentally, the relative number of proteins can be quantified in living cells using fluorescent proteins (Elowitz *et al*, 2002; Ozbudak *et al*, 2002; Blake *et al*, 2003; Raser and O'Shea, 2004). Denoting I_1 as the intrinsic variable (the number of proteins) for the first copy of the system and I_2 the equivalent for the second copy, then intrinsic noise is determined by a measure of the difference between I_1 and I_2 because intrinsic fluctuations cause variation in I_1 to be uncorrelated with that of I_2. Extrinsic fluctuations, however, cause variation in I_1 and I_2 to be correlated because they equally affect both copies of the system. Extrinsic noise is a measure of this correlation and is determined by the cross-correlation function of I_1 and I_2. The squares of the intrinsic and the extrinsic noise sum to give the square of the total noise of the intrinsic variable, which is defined as its coefficient of variation (Swain *et al*, 2002).

Here, we consider the effects of extrinsic fluctuations on biochemical networks. Extrinsic fluctuations typically cause fluctuations in the parameters of a network (Paulsson, 2004). For example, Figure 1A shows a model of gene expression that includes promoter activation, transcription, translation, and degradation (Kepler and Elston, 2001; Raser and O'Shea, 2004; Golding *et al*, 2005; Kaern *et al*, 2005). In this model, v_1 is the rate of translation. It is a function of the number of free ribosomes, an extrinsic variable, and will fluctuate as the number of free ribosomes changes. Extrinsic fluctuations have an average lifetime that is not zero (they are 'colored') (Rosenfeld *et al*, 2005). We will show that this extrinsic timescale can profoundly affect the system's dynamics and stochastic properties. It can determine the lifetime of protein fluctuations and change mean protein numbers. Extrinsic fluctuations being nonspecific can act simultaneously on many parameters of the network. This nonspecificity can cause fluctuations to combine constructively or destructively, dramatically altering the network's output. For our simulations, we designed a novel extension of the standard algorithm for simulating intrinsic fluctuations (Gillespie, 1976) that includes discontinuous, time-varying parameters and therefore can also simulate extrinsic fluctuations with any desired properties (Materials and methods).

Results

Extrinsic fluctuations alter mean protein numbers and intrinsic noise

Extrinsic fluctuations can substantially change the distribution of protein numbers. Figure 1B shows the steady-state distribution of protein numbers for the model of Figure 1A with no extrinsic fluctuations. It is slightly asymmetric and is expected to approximate a gamma distribution (Friedman *et al*, 2006). Figure 1C shows the corresponding joint probability distribution of I_1 and I_2. Although the system is generally described by a probability distribution that includes all the intrinsic variables for the first copy of the network, all the intrinsic variables for the second copy, and all the extrinsic variables, a projection of this distribution onto I_1 and I_2 is sufficient for calculating noise (Elowitz *et al*, 2002). With no extrinsic fluctuations, the distribution spreads parallel to the I_1 and I_2 axes (Figure 1C): I_1 and I_2 are independent and have no correlation ($\eta_{ext}=0$). With colored extrinsic fluctuations, the mode and mean of the distribution of protein numbers can change, its variance increases, and there can be a longer tail at high numbers (Figure 1D). Correspondingly, the probability distribution for I_1 and I_2 spreads along the line $I_1=I_2$: I_1 and I_2 are now correlated through fluctuations in the extrinsic variable (here, the rate k_0: Figure 1D inset). Larger extrinsic fluctuations would cause the distribution to spread along and tighten around the line $I_1=I_2$. Larger intrinsic fluctuations would cause the distribution to expand away from the line.

Changing the properties of extrinsic fluctuations—their source, magnitude, and typical lifetime (τ)—can alter mean protein numbers and measurements of the intrinsic noise. The effect of extrinsic fluctuations is determined by both their coefficient of variation and their lifetime. Extrinsic fluctuations with lifetimes shorter than a cell cycle could be generated by negative feedback or fast protein degradation; those longer than the cell cycle could be generated by positive feedback (Cox *et al*, 2006). As the coefficient of variation of any parameter in Figure 1A increases, the extrinsic noise measured in protein numbers increases (Figure 2A). Similarly, as the lifetime of extrinsic fluctuations increases, the extrinsic noise increases: extrinsic fluctuations that are fast compared to intrinsic fluctuations are averaged away and contribute little to the extrinsic noise (Figure 2B). If the extrinsic fluctuations occur in a parameter that determines the lifetime of fluctuations in protein numbers, such as the protein degradation rate, then the extrinsic timescale mixes with the intrinsic timescales and the mean (and the mode) of the protein distribution can shift (Figure 2C and D). Although this change in mean protein numbers implies that extrinsic fluctuations can change measurements of intrinsic noise, the change we observe is more than expected (Figure 2E and F): for example, if the translation rate, v_1, fluctuates, the mean protein number changes little, but there is over a two-fold increase in intrinsic noise.

To understand how extrinsic fluctuations affect intrinsic noise, consider only the measured intrinsic variables and one extrinsic variable, E say. The system can then be described by the probability distribution $P(I_1,I_2,E)$. Changing the properties of the extrinsic variable will alter extrinsic fluctuations and the shape of this three-dimensional distribution. Colored extrinsic fluctuations imply that $P(I_1,I_2,E)=P(I_1|I_2,E)P(I_2|E)P(E)$ only, and not $P(I_1,I_2,E)=P(I_1|E)P(I_2|E)P(E)$, because the current value of I_2 contains predictive information on the history of E over the timescale associated with I_2 (typically a protein lifetime). Extrinsic and intrinsic variables are strongly co-dependent. Changing the shape of $P(I_1,I_2,E)$ by altering extrinsic fluctuations can potentially change its projection onto the I_1 and I_2 planes (Figure 1C and E). The intrinsic noise, which is determined by the I_1 and I_2 projection, can therefore vary with extrinsic fluctuations.

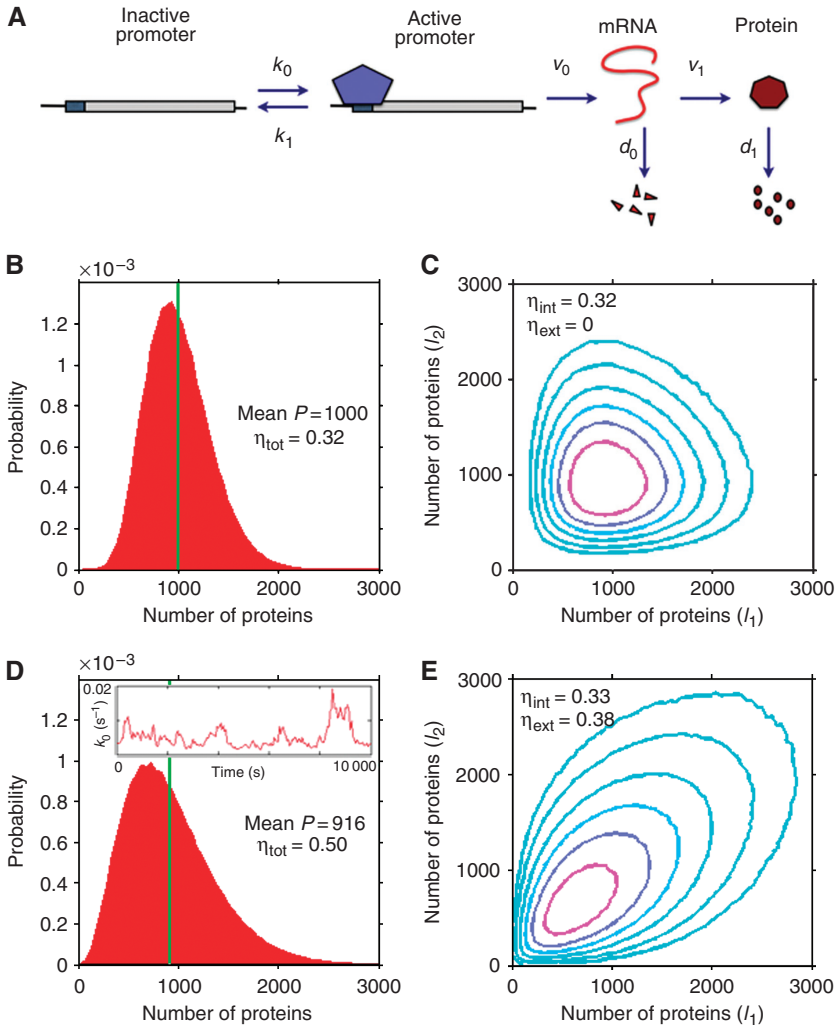

Figure 1 Extrinsic fluctuations change substantially the probability distribution for protein numbers during gene expression. (**A**) A model of gene expression with two states of the promoter, one active, and able to initiate transcription, and the other inactive. We have shown the binding of RNA polymerase (purple pentagon) driving the transition from inactive to active, but the transition may occur through the binding of transcription factors or changes in the structure of chromatin (Blake *et al*, 2003; Raser and O'Shea, 2004; Golding *et al*, 2005). Once active, the promoter can initiate transcription on average every $1/v_0$ seconds and synthesize mRNA which in turn is translated into protein on average every $1/v_1$ seconds. Both protein and mRNA undergo first-order degradation. We use parameters appropriate for *Escherichia coli* (Golding *et al*, 2005): k_0=0.005 s^{-1}, k_1=0.03 s^{-1}, v_0=0.07 s^{-1}, d_0=0.005 s^{-1}, v_1=0.2 s^{-1}, and d_1=0.0004 s^{-1}. The longest intrinsic timescale is then 2500 s, the promoter is active approximately 15% of the time, and the mean steady-state number of proteins is 1000. (**B**) A histogram of protein numbers generated by stochastic simulation of (A). Only intrinsic fluctuations are included. The distribution is slightly skewed with the mode close to the mean, which is shown by the green line. (**C**) A contour plot of the joint protein probability distribution generated by a two-color experiment for which we simulate two identical copies of the system. (**D**) A histogram of protein numbers generated from intrinsic fluctuations and a fluctuating extrinsic variable: k_0, the probability per unit time of the promoter transitioning from the inactive to the active state. The inset shows typical variation of k_0. Extrinsic fluctuations are generated by a log-normal stochastic process with an autocorrelation time of approximately 10^3 s: the mean of k_0 is unchanged and it has a coefficient of variation of 1. The measured extrinsic noise in protein levels is then approximately 0.4. (**E**) The corresponding joint probability distribution for I_1 and I_2 in a two-color simulation.

Mathematically, extrinsic fluctuations affect the dynamics as multiplicative noise and the system of Figure 1A becomes nonlinear. We verified the results of Figure 2 using both a Langevin approach (van Kampen, 1990; Swain, 2004), which is suitable for many fluctuating variables but is a linear approximation, and the unified colored noise approximation (Jung and Hanggi, 1987), which is nonlinear but suitable for only one fluctuating variable. Both the shifting of the mean protein number and the changes in intrinsic noise are nonlinear effects of colored extrinsic fluctuations and are not reproduced by Langevin theory (Figure 2 and Supplementary information).

Our approach also provides a general technique for stochastic sensitivity analysis because we apply fluctuations to parameters of the system (Stelling *et al*, 2004). We can therefore determine, for example, the robustness of the concentration of the network output or any other network property to changes in parameter values. Sensitive parameters generate both a high intrinsic and a high extrinsic noise (a high total noise) in the property under investigation. Within our model, we predict that protein levels are most sensitive to fluctuations in the transcription and translation rates, v_0 and v_1 (Figure 2A and E).

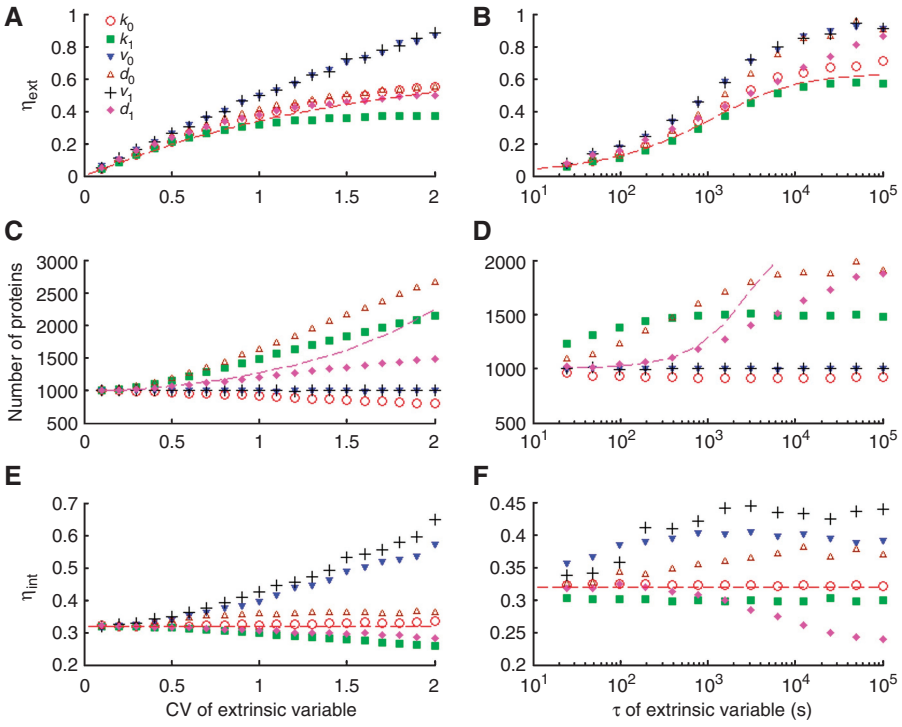

Figure 2 The effects of the magnitude of extrinsic fluctuations (the coefficient of variation) and their duration (the autocorrelation time τ) on measurements of the extrinsic noise (**A**, **B**), mean protein numbers (**C**, **D**), and the intrinsic noise (**E**, **F**) for the gene expression of Figure 1A. We simulate fluctuations in either k_0 (orange circles), k_1 (green squares), v_0 (blue triangles), d_0 (red triangles), v_1 (black crosses), or d_1 (purple diamonds). When the coefficient of variation of the extrinsic variable changes, τ is 10^3 s (A, C, E). When τ varies, the coefficient of variation is 1 (B, D, F). Each simulation data point is calculated from 10^8 s of simulation. The dashed lines are analytical solutions using the unified colored noise approximation (applied to d_1; C, D) or Langevin theory (applied to v_0; A, B, E, F).

Extrinsic fluctuations can help describe trends in high-throughput measurements

We can use these properties of extrinsic fluctuations to explain high-throughput measurements of stochasticity. Total noise in protein numbers scales with the inverse square root of mean protein number (Bar-Even *et al*, 2006; Newman *et al*, 2006). This relationship is fundamentally a property of intrinsic fluctuations (Bar-Even *et al*, 2006), but still holds with substantial extrinsic fluctuations because extrinsic fluctuations can change mean protein numbers and intrinsic noise (Figure 3A). To test this proposal, we simulated many models with different parameter sets, each generated from the scheme of Figure 1A by log-normal sampling (Materials and methods). Our results imply that the high-throughout measurements are consistent with other studies, which shows extrinsic noise to be dominant (Elowitz *et al*, 2002; Raser and O'Shea, 2004).

Extrinsic fluctuations can cause correlations between the lifetime of protein fluctuations and the extrinsic noise in protein levels, if they have the dominant timescale of the system. The lifetime of protein fluctuations will then be determined by the lifetime of the extrinsic fluctuations. With the same set of simulations, we measure a significant correlation between the timescale of protein fluctuations and total noise (Figure 3B). It arises because many of the models we simulate have extrinsic noise greater than intrinsic noise. The correlation is also evident in Figure 2B. Indeed, time series studies in human cells have shown that the total noise to be correlated with the autocorrelation time of protein levels (Sigal *et al*, 2006).

Extrinsic fluctuations can affect the performance of genetic networks

We next considered the effect of extrinsic fluctuations on one of the simplest regulatory networks: a negatively auto-regulated loop. Experiments suggest that stochasticity is attenuated by negative auto-regulation, at least for a plasmid-borne system (Becskei and Serrano, 2000). Negative feedback reduces fluctuations by increasing expression when protein numbers are low and decreasing expression when protein numbers are high. Increasing the strength of the feedback increases its potential to reduce stochasticity (Thattai and van Oudenaarden, 2001; Simpson *et al*, 2003; Swain, 2004), but decreases the copy numbers of mRNAs and proteins. The corresponding rise in intrinsic fluctuations may surpass any attenuation (Figure 4A and B) (Tan *et al*, 2007). Extrinsic noise is mostly independent of protein numbers. It will therefore decrease with the addition of negative feedback. Consequently, the total noise of a constitutive system to which auto-negative feedback is added can either increase if intrinsic fluctuations dominate (Figure 4A) or decrease if extrinsic fluctuations dominate (Figure 4B). Consistently, experiments show a range of auto-repression strength for which noise minimization is optimal, although this observation was

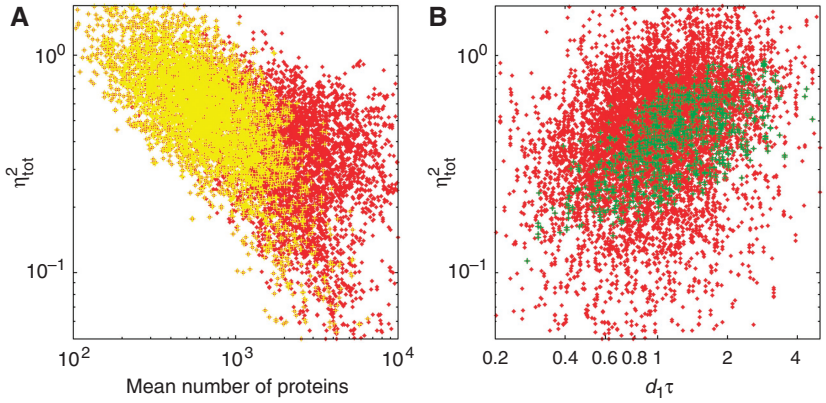

Figure 3 Correlations between the total noise in protein numbers and mean protein numbers and the total noise in protein numbers and the timescale of the extrinsic fluctuations. We randomly generated 10 000 sets of parameters for the model of gene expression in Figure 1A. We added extrinsic fluctuations to one randomly chosen parameter of the model (Materials and methods). Overall, the mean ratio of extrinsic noise measured in protein numbers to intrinsic noise is about 2. (**A**) The square of the total noise and the mean protein number have a negative correlation of approximately 0.4 for the entire data set (red points and yellow crosses). For parameters where the measured intrinsic noise is at least 40% of the total noise (yellow crosses), the magnitude of this correlation increases to approximately 0.6, which is comparable to that observed experimentally (Newman *et al*, 2006). There is also a correlation of approximately 0.4 between the intrinsic and the extrinsic noise. (**B**) The square of the total noise and the timescale of the extrinsic fluctuations measured in the units of protein lifetime (τd_1) have a correlation coefficient of approximately 0.3 for the entire data set (red points and green crosses). For parameter sets with extrinsic noise at least 75% of the total noise (green crosses), this correlation increases to 0.6, which is comparable to that observed experimentally (Sigal *et al*, 2006).

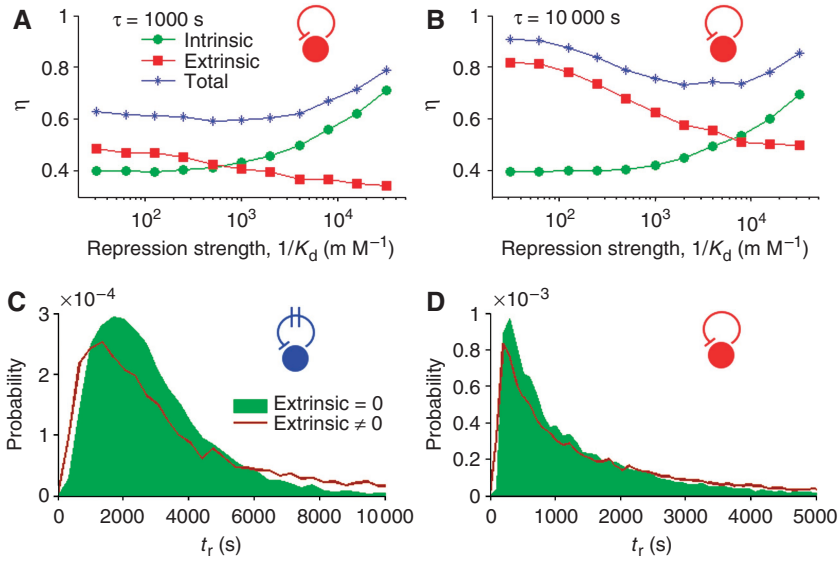

Figure 4 Extrinsic fluctuations can enhance the effects of negative auto-regulation on stochasticity and response times. Proteins are repressors and can bind to the inactive promoter state in Figure 1A with a dissociation constant K_d. We let v_0 have extrinsic fluctuations with a coefficient of variation of 1. The intrinsic noise increases and the extrinsic noise decreases as the strength of the feedback increases. The steady-state number of proteins drops from 1000 with no feedback to 300 when the feedback is maximum. (**A**) For extrinsic fluctuations with $\tau=10^3$ s, the total noise mostly increases with feedback strength. (**B**) For extrinsic fluctuations with $\tau=10^4$ s, the total noise mostly decreases with feedback strength. In both cases, there is an optimum K_d for which the intrinsic and extrinsic noise are equal, and the total noise is minimum. (**C**) The response time distribution for constitutive expression: measuring from the initiation of transcription, t_r is the time taken to first reach 63% of the mean steady-state number of proteins. Extrinsic fluctuations ($\tau=10^4$ s) decrease the mode of the distribution from 1800 to 1300 s, while the mean increases from 2900 to 4700 s. (**D**) The response time distribution for an auto-negative system ($K_d \simeq 60$ nM). Extrinsic fluctuations ($\tau=10^4$ s) decrease the mode from 300 to 200 s. The mean increases from 1300 to 1900 s. Negative feedback reduces the mean t_r by a factor of $\simeq 2$ and the mode t_r by a factor of $\simeq 6$.

attributed to plasmid variation (Dublanche *et al*, 2006). Our results suggest extrinsic fluctuations in any parameter of the system could create the same effect. We also predict that negative feedback is more likely to evolve as an attenuator of stochasticity in systems dominated by extrinsic fluctuations (Paulsson, 2004; Hooshangi and Weiss, 2006). Alternatively, intrinsic fluctuations could be reduced by an additional positive feedback loop to maintain high protein copy numbers despite the negative feedback needed to attenuate extrinsic fluctuations. For example, positive and negative feedbacks occur in the GAL regulon in budding yeast and have been shown to reduce measurements of noise (Ramsey *et al*, 2006).

Negative auto-regulation also reduces response times (Savageau, 1974; Rosenfeld *et al*, 2002). The mean time for an auto-negative system to reach 63%, or $1-e^{-1}$, of its steady-state number of proteins from the initiation of transcription, t_r, is reduced by at least a factor of two by negative feedback (Figure 4C and D). This reduction occurs because negative feedback decreases the timescales of the system and so shifts the power spectrum of a constitutively expressed gene to higher frequencies (Simpson *et al*, 2003; Austin *et al*, 2006). Intuitively, negative feedback initially allows expression at a rate higher than the steady-state rate of expression while the first repressors are synthesized (Rosenfeld *et al*, 2002). Stochastic fluctuations can cause significant variation in timing (Amir *et al*, 2007), and we observe that the probability distribution of t_r is asymmetric and the asymmetry is enhanced by extrinsic fluctuations (Figure 4C and D). An extrinsic fluctuation can either aid or inhibit gene expression, and its substantial lifetime ensures that such effects contribute significantly to t_r. Despite increasing the mean response time, extrinsic fluctuations enable cells to typically respond faster, irrespective of negative feedback, because the most probable t_r decreases (Figure 4C and D). Yet a population of cells can better 'hedge its bets' because a greater number will rarely respond: the distribution has a longer tail for high response times.

Extrinsic fluctuations can combine destructively and constructively

Extrinsic fluctuations are nonspecific: they can act simultaneously on many parameters of a network (Pedraza and van Oudenaarden, 2005). Nonspecific extrinsic fluctuations could arise, for example, from changes in the cell's growth rate or its environment. We added extrinsic fluctuations to all pairs of parameters in the model of Figure 1A. These fluctuations were either uncorrelated, and generated by individual sources of stochasticity, or identical, and generated by the same stochasticity source (Figure 5A and B). For uncorrelated extrinsic fluctuations, the extrinsic fluctuations in each parameter combine constructively: the square of the extrinsic noise is approximately the sum of the squares of the extrinsic noises generated when each parameter fluctuates alone (Figure 5A). For identical or, more generally, correlated extrinsic fluctuations, the extrinsic fluctuations in each parameter also combine constructively if both parameters affect protein numbers similarly (protein numbers are proportional or inversely proportional to both parameters). Extrinsic fluctuations can be destructive, however, if both parameters have opposing effects on protein numbers (protein numbers are proportional to one parameter and inversely proportional to the other). Fluctuations in the two extrinsic variables then have little effect on measurements of the extrinsic noise because a fluctuation in the variable that acts to increase protein numbers is counteracted by the same, or a similar, fluctuation in the variable that acts to decrease protein numbers (Figure 5B). A network architecture that channels extrinsic fluctuations into two parameters with opposing effects on protein numbers can therefore attenuate stochasticity, and one that channels extrinsic fluctuations into two parameters with similar effects on protein numbers can be a stochasticity amplifier. We confirmed these results using a Langevin calculation (Supplementary information).

Constructive and destructive extrinsic fluctuations occur in feedforward loops, one of the most common motifs in genetic networks (Milo *et al*, 2002). Figure 5C and D illustrates two feedforwards, where gene *Z* is activated by genes *X* and *Y*, and gene *Y* is either activated by gene X (coherent feedforward) or repressed by gene *X* (incoherent feedforward) (Mangan and Alon, 2003). Extrinsic fluctuations in *X* proteins generate fluctuations in *Y* proteins because *X* regulates *Y*. Feedforwards channel these correlated fluctuations in *X* and *Y* into the levels of *Z* proteins because both *X* and *Y* regulate *Z*. Fluctuations can combine constructively or destructively at *Z*. If the timescale of the extrinsic fluctuations is less than intrinsic timescales, however, extrinsic fluctuations are averaged away and such effects are no longer seen (Ghosh *et al*, 2005; Hayot and Jayaprakash, 2005). In the coherent feedforward loop, the extrinsic fluctuations combine constructively because *X* and *Y* affect gene expression of *Z* similarly (Figure 5C). In the incoherent feedforward loop, *X* and *Y* have opposing affects on gene expression and their extrinsic fluctuations combine destructively (Figure 5D). As well as being sign-sensitive delays and accelerators (Mangan and Alon, 2003), feedforward loops may therefore also have been selected to amplify or attenuate extrinsic fluctuations in their input, *X*.

Discussion

Here, we have extended the standard stochastic simulation algorithm for simulating intrinsic fluctuations in biochemical networks (Gillespie, 1976) to include extrinsic fluctuations (Materials and methods). Although extrinsic fluctuations have been modeled previously (Austin *et al*, 2006; Cox *et al*, 2006; Geva-Zatorsky *et al*, 2006; Scott *et al*, 2006; Sigal *et al*, 2006; Tanase-Nicola *et al*, 2006; Tsimring *et al*, 2006; Volfson *et al*, 2006; Maithreye and Sinha, 2007), our approach is more general: we can simulate extrinsic fluctuations with any desired properties; we can vary many parameters with correlated or uncorrelated fluctuations; and we are able to average over intrinsic fluctuations by repeating simulations with the same trajectory of extrinsic variation. In the Supplementary information, we show that time-varying extrinsic fluctuations lead to a generalization of the original interpretations of intrinsic and extrinsic noise (Swain *et al*, 2002).

Both the magnitude and the timescales of fluctuations are necessary to predict the effects of one stochastic system interacting with another. The mixing of the timescales of the two systems through their interaction can lead to so-called deviant effects (Samoilov and Arkin, 2006), such as a shifting of the mean and asymmetries in the distribution of protein numbers. Extrinsic fluctuations can even decrease the intrinsic noise measured in protein levels. We predict that deviant effects will be common in biochemical networks because these networks typically have substantial extrinsic fluctuations and the timescale of these fluctuations can be the longest timescale in the system. Indeed, such effects are present in high-throughput measurements of cellular variation (Bar-Even *et al*, 2006; Newman *et al*, 2006; Sigal *et al*, 2006).

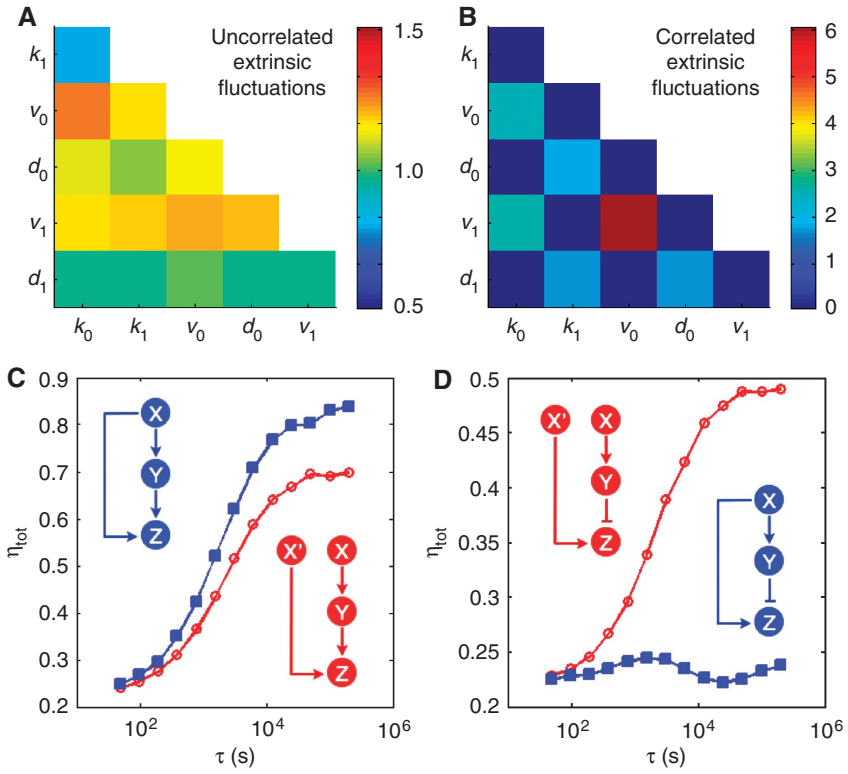

Figure 5 Nonspecific effects of extrinsic fluctuations: fluctuations can combine constructively or destructively when applied to two parameters in Figure 1A. Let $\eta_{ext}^{(i)}$ be the extrinsic noise measured when the parameter *i* fluctuates, and let $\eta_{ext}^{(i,j)}$ be the extrinsic noise measured when parameters *i* and *j* fluctuate. (**A**) The relative extrinsic noise, $(\eta_{ext}^{(i,j)})^2/[(\eta_{ext}^{(i)})^2+(\eta_{ext}^{(j)})^2]$, when uncorrelated extrinsic fluctuations are applied to a pair of parameters. The scale bar shows the magnitude of the relative extrinsic noise: $(\eta_{ext}^{(i,j)})^2 \simeq (\eta_{ext}^{(i)})^2+(\eta_{ext}^{(j)})^2$. (**B**) The relative extrinsic noise when correlated extrinsic fluctuations are applied to a pair of parameters: $(\eta_{ext}^{(i,j)})^2 \gg (\eta_{ext}^{(i)})^2+(\eta_{ext}^{(j)})^2$ or $(\eta_{ext}^{(i,j)})^2 \ll (\eta_{ext}^{(i)})^2+(\eta_{ext}^{(j)})^2$. Note the different scale bar. (**C**) The total noise measured in a coherent feedforward loop versus the timescale of extrinsic fluctuations in the number of *X* proteins. The feedforward amplifies stochasticity: extrinsic fluctuations in *X* combine constructively with those in *Y*. (**D**) The total noise in an incoherent feedforward loop versus the timescale of extrinsic fluctuations in the number of *X* proteins. The feedforward attenuates stochasticity: extrinsic fluctuations in *X* combine destructively with those in *Y*. In (C, D), results for the feedforward loop are shown with blue squares and results for the equivalent 'open' loop are shown with red circles. In the open loop, *X*′ has the same mean copy number as *X*, but uncorrelated extrinsic fluctuations. The coefficient of variation of the extrinsic fluctuations in *X* or *X*′ is 1. The two protein inputs *X* and *Y* regulate the gene *Z* through an AND gate. Other types of feedforward or regulation give similar results.

We can use our simulation method to investigate the source of extrinsic fluctuations. Interpreting our results as a stochastic sensitivity analysis, we predict that variation in transcription and translation rates to be the most significant sources. Such variation could arise from fluctuations in the numbers of free ribosomes or RNA polymerases or in the numbers of ribonucleotides, tRNAs, or amino acids. Being based on the parameter set of Figure 1A, this prediction is model specific, but we expect it to hold for other genes in *Escherichia coli*.

Extrinsic fluctuations can create stochasticity in the output of a network of a magnitude that is substantially different from the magnitude of the extrinsic fluctuations themselves. If correlated, fluctuations in two parameters of a network can combine constructively to create extrinsic noise in the protein output that is many times the extrinsic noise measured for each parameter fluctuating independently. A different network architecture, however, can cause correlated extrinsic fluctuations to almost entirely negate each other. Both effects are likely to be present in cells. We predict that constructive and destructive extrinsic fluctuations are present in feedforward loops in genetic networks, and Tanase-Nicola *et al* (2006) predict destructive fluctuations in ultra-sensitive protein signaling cascades.

Extrinsic fluctuations, through their timescales and non-specificity, are thus an important component of the intracellular environment. To function in this environment, biochemical networks are likely to have evolved to control or exploit these fluctuations. Our stochastic simulation algorithm and mathematical analysis should therefore help to quantitatively understand endogenous networks and to design effective synthetic ones.

Materials and methods

To simulate extrinsic fluctuations, we extend Gillespie's first reaction algorithm (Gillespie, 1976) to include discontinuous, time-dependent reaction rates. In the first reaction algorithm, a putative time for each potential reaction in the system is calculated, and the reaction whose putative time is first is implemented. Simulation time is then incremented by this reaction time. Each putative reaction time is calculated from the propensity of the reaction: the probability of the reaction per unit time multiplied by all ways of selecting the reactants (Gillespie, 1976). The propensity, $a(t)$, is a function of time if the probability of the reaction per unit time is not constant.

For a time-varying propensity, we can show (Supplementary information) that the putative reaction time, τ, obeys

$$\int_0^{\tau} \mathrm{d}t\, a(t) = \log(1/r) \qquad (1)$$

where r is a uniform random number between 0 and 1. Equation (1) is general, but it may be difficult to analytically find τ for a complex $a(t)$. Consequently, we approximate $a(t)$ by a series of step functions or a piecewise-linear function. If we sample $a(t)$ every Δt seconds and use the more accurate piecewise linear approximation, then $a(t) \sim a_0 + a_1 t$ for t within a Δt interval. Here, a_0 and a_1 are constants defined by the Taylor series of $a(t)$ and will change discontinuously from one Δt interval to the next. We can use equation (1) to exactly implement discontinuous changes in a propensity (Supplementary information). Briefly, if the next predicted reaction would bring the simulation time into the next Δt interval, we do not implement this reaction, but instead change the time-dependent propensity to the new functional form valid for the new Δt interval. We set the simulation time to the start of the new Δt interval and re-calculate the putative reaction times for all the reactions.

To calculate the putative reaction time within each Δt interval when $a(t)=a_0+a_1 t$, we again use equation (1) which implies

$$\tau = \frac{a_0}{a_1}\left(\sqrt{1 - \frac{2a_1}{a_0^2}\log(r) - 1}\right) \qquad (2)$$

where τ obeys $0 \leqslant \tau \leqslant \Delta t$. If $a_1 < 0$ and $r > \mathrm{e}^{\frac{a_0^2}{2a_1}}$, then the reaction cannot occur ($\tau = \infty$).

By generating a time series for a source of extrinsic fluctuations before running our algorithm, we can then use this time series to change reaction rates appropriately to simulate extrinsic fluctuations. We use the Ornstein–Uhlenbeck process to generate the time series (Fox *et al*, 1988; Gillespie, 1992). This process, $\varepsilon(t)$, has a positive autocorrelation time and is normally distributed. Consequently, when added to a parameter k so that $k \to k + \varepsilon(t)$, k can become negative. Exponentiating $\varepsilon(t)$, however, and letting $k \to k\mathrm{e}^{\varepsilon(t)}/\langle \mathrm{e}^{\varepsilon(t)} \rangle$ generates a log-normal stochastic process for k. Such a process is suitable for modeling fluctuations in extrinsic variables (Rosenfeld *et al*, 2005): k has a fixed mean, a finite auto-correlation time, and is always positive.

When measuring extrinsic and intrinsic noise, we simulate two copies of the model of Figure 1A. We define

$$\eta_{\mathrm{int}}^2 = \frac{\langle (I_1 - I_2)^2 \rangle}{2\langle I \rangle^2};\ \eta_{\mathrm{ext}}^2 = \frac{\langle I_1 I_2 \rangle - \langle I \rangle^2}{\langle I_1 \rangle^2} \qquad (3)$$

where I_1 is the number of proteins for the first copy and I_2 the number of proteins for the second copy. We use $\langle I_1 \rangle = \langle I_2 \rangle = \langle I \rangle$ because both copies have the same mean. We simulate with the Gibson and Bruck (2000) version of the Gillespie method using the *Facile* network complier and its stochastic simulator *EasyStoch* (Siso-Nadal *et al*, 2007). Both are freely available at www.cnd.mcgill.ca/~swain. All reactions and kinetic rates are included in the Supplementary information. Averages are time averages taken over many times the longest timescale of the system.

For Figure 3, we generated parameter sets for our model of gene expression from log-normal distributions with means given by the parameter values in Figure 1A and a standard deviation in log-space of 20% of the mean. We choose k_0, however, by sampling the probability of the promoter being in the active state from a log-normal distribution with a mean given by the parameters in Figure 1A and with a standard deviation of 70% of this mean in log-space. We let extrinsic fluctuations act on a randomly chosen parameter in each model. We chose the coefficient of variation of these fluctuations from a normal distribution with a mean of 1 and a standard deviation of 0.1. For each model, we sample τ from a log-normal distribution with a mean of 2500 s, the mean protein lifetime, and with a standard deviation of 50% of this mean in log-space.

Supplementary information

Supplementary information is available at the Molecular Systems Biology website (www.nature.com/msb).

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Conflict of interest

The authors declare there is no financial conflict.

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