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Abstract

Background: Gene expression in a cell entails random reaction events occurring over disparate time scales. Thus, molecular noise that often results in phenotypic and population-dynamic consequences sets a fundamental limit to biochemical signaling. While there have been numerous studies correlating the architecture of cellular reaction networks with noise tolerance, only a limited effort has been made to understand the dynamical role of protein-protein associations.

Results: We have developed a fully stochastic model for the positive feedback control of a single gene, as well as a pair of genes (toggle switch), integrating quantitative results from previous *in vivo* and *in vitro* studies. In particular, we explicitly account for the fast protein binding-unbinding kinetics, RNA polymerases, and the promoter/operator sequences of DNA. We find that the overall noise-level is reduced and the frequency content of the noise is dramatically shifted to the physiologically irrelevant high-frequency regime in the presence of protein dimerization. This is independent of the choice of monomer or dimer as transcription factor and persists throughout the multiple model toplogies considered. For the toggle switch, we additionally find that the presence of a protein dimer, either homodimer or heterodimer, may significantly reduce its intrinsic switching rate. Hence, the dimer promotes the robust function of bistable switches by preventing the uninduced (induced) state from randomly being induced (uninduced).

Conclusions: The specific binding between regulatory proteins provides a buffer that may prevent the propagation of fluctuations in genetic activity. The capacity of the buffer is a non-monotonic function of association-dissociation rates. Since the protein oligomerization *per se* does not require extra protein components to be expressed, it provides a basis for the rapid control of intrinsic or extrinsic noise. The stabilization of phenotypically important toggle switches, and nested positive feedback loops in general, is of direct implications to organism fitness. Finally, noise control through oligomerization suggests avenues for the design of robust synthetic gene circuits for engineering purposes.

Background

Recent experiments on isogenic populations of microbes with single-cell resolution [1–3] have demonstrated that stochastic fluctuations, or noise, can override genetic and environmental determinism. In fact, the presence of noise may significantly affect the

fitness of an organism [4]. The traditional approach for modeling the process of molecular synthesis and degradation inside a cell is by deterministic rate equations, where the continuous change of arbitrarily small fractions of molecules is controlled instantaneously and frequently represented through sigmoidal dose-response relations. However, the rate-equation approaches can not explain the observed phenotypic variability in an isogenic population in stable environments. In particular, when molecules involved in feedback control exist in low copy numbers, noise may give rise to significant cell-to-cell variation as many regulatory events are triggered by molecules with very low copy numbers $\lesssim 100$ [5]. A well known example is the regulation of inorganic trace elements [6], such as iron, copper, and zinc. While these trace elements are essential for the activity of multiple enzymes, their presence may quickly turn cytotoxic unless their concentrations are carefully controlled.

Although the presence of phenotypic variation due to stochastic fluctuations need not be detrimental for a population of cells [7], elaborate regulatory mechanisms have evolved to control the effects of noise. Several systems-biology studies have recently focused on a select set gene-regulatory circuits, in particular those with feedback control. Feedback control circuits have been identified as important for multiple species and proven responsible for noise reduction and increased functional stability in many housekeeping genes through negative autoregulation [8], long cascades of ultrasensitive signaling [9], bacterial chemotaxis [10], and the circadian clock [11]. Additionally, recent studies on iron homeostasis [12, 13] in *E. coli* highlight the noise-reducing capability mediated by small RNAs.

Here, we study the stabilizing effect on noisy positive-feedback circuits (PFCs) by the presence of oligomerized transcription factors. We have analyzed the effect of protein oligomerization on noise in positive-feedback autoregulatory circuits as well as a simple toggle-switch [14]. The all-or-none threshold behavior of PFCs typically improves robustness against "leaky" switching. However, due to their functional purpose, gene circuits involved in developmental processes or stress responses that often accompany genome-wide changes in gene expression are intrinsically more noisy than basic negative feedback circuits.

It is frequently observed that transcription factors exist in oligomeric form [15], and protein oligomerization is an important subset of protein-protein interactions, costituting a recurring theme in enzymatic proteins as well as regulatory proteins. Well studied examples include the λ-phage repressor, λCI (dimer), the TrpR (dimer), LacR (tetramer), and Lrp (hexadecamer or octamer). While many of the RNA-binding proteins dimerize exclusively in the cytosol, the LexA repressor [16], the leucine-zipper activator [17, 18], and the Arc repressor [19] have been shown to form an oligomer either in the cytosol ("dimer path") or on the DNA by sequential binding ("monomer path"). Previously, the efficacy of monomer and dimer transription-regulation paths to reduce noise was separately studied for a negative-feedback autoregulatory circuit [20]. In contrast, we have focused on oligomerization in positive-feedback autoregulatory circuits, as well as a simple positive-feedback toggle-switch [**?**]. We find that cytosolic transcription-factor oligomerization acts as a significant buffer for abundance-fluctuations in the monomer, overall reducing noise in the circuit. Additionally, the noise-power spectral density is shifted from the low- to the high-frequency regime. In the toggle switch, cytosolic oligomerization in the transcription factor may significantly stabilize the functional state of the circuit.

Although our modeling and analysis is based on prokaryotic cells, we expect our main findings to be organism-independet since protein oligomers, especially homodimers, is such a common occurrence across the species [21], with homodimers comprising 12.6% of the high-fidelity human proteome [22, 23].

Results and Discussion

Dimerization breaks long-time noise correlations in autogenous circuit

To evaluate the dynamic effects of protein-protein binding in positive-autoregulation gene circuits, we construct several alternative models of positive autogenous circuits. Each model emphasizes a different combination of possible feedback mechanisms, and the network topologies considered can be grouped into the two classes of monomer-only (MO) and dimer-allowed (DA) circuits, according to the availability of a protein-dimer state (Fig. 1). We further group the DA circuits into three variations, DA1 through DA3, depending on which form of the protein is the functional transcription factor (TF) and where the dimerization occurs. For DA1, we only

allow the dimer to bind with the DNA-operator sequence, while for DA2 dimerization occurs through sequential binding of monomers on the DNA. In DA3, the protein-DNA binding kinetics are the same as in the MO circuit with the addition of dimerization in the cytosol. While we will only present results for DA1 in this paper, there is no significant difference for DA2 and DA3 (see *Supplementary Material*).

Note that the feedback loop (the auto-regulation) that is implicit in Fig. 1 is included through the dependence of the RNAp-DNA binding rates on the presence or absence of the protein (TF)-operator complex. The sign (positive or negative) of the feedback is a consquence of the relative binding strength between DNA and RNAp in the presence and absence of the transcription-factor-operator complex. For instance, if $K_{30} = k_{30}/q_{30} > K_{32} = k_{31}/q_{31}$, the control is that of positive feedback. For each topology, we study the dependence of noise characteristics on the kinetic rates by varying the dimer half-life, binding affinity, and the individual association/dissociation rates. While we only discuss positive feedback control of the autogenous circuit in this paper, we have obtained corresponding results for negative feedback control.

Figure 2 shows a sample of ten representative time courses for the protein abundance using the DA1 topology. The effect of stochastic fluctuations is marked in the MO circuit. However, in all the DA circuits where the protein may form a cytosolic dimer we observe a significantly reduced noise level for the monomer abundance. The suppression of fluctuations persists throughout the range of kinetic parameters that (so far) is known to be physiologically relevant (Table 1).

Calculating the steady-state distribution for the monomer and dimer abundances (Fig. 3) we observe a clear trend that the monomer Fano factor ($\nu = \sigma^2/\mu$) is reduced as the binding equilibrium is shifted towards the dimer. This trend is conserved for all the investigated DA topologies (see *Supplementary Information* for other circuit topologies). As long as dimerization is allowed in the cytosol, the fast-binding equilibrium absorbs long-time fluctuations stemming from bursty synthesis or decay of the monomer. When a random fluctuation brings about a sudden change in the monomer copy number, dimerization provides a buffering pool that absorbs the sudden change. Otherwise, random bursts in the monomer abundance will propagate to the transcriptional activity of the promoter, leading to erratic control of protein expression. It should be emphasized that this has nothing to do with the sign of regulation and is in agreement with the observations of Ref. [20] for negative autoregulation. Surprisingly, the magnitude of noise reduction in the positive autoregulatory circuit is nearly the same as that for negative autoregulation which is typically considered a highly stable construct (see *Supplementary Information*).

A heuristic explanation is given by the linear response theory of dynamical system, which is justified for small perturbations around a steady state. When a random fluctuation shifts the monomer copy number away from its steady-state value, the decay toward the steady state can be described by the system Jacobian. The disparity in the magnitude of the (negative) eigenvalues of the Jacobian matrix for the MO versus the DA circuits signifies the fact that the perturbed state is buffered by fast settlement of the monomer-dimer equilibrium before random fluctuation can accumulate, possibly with catastrophic physiological effects. This explains the coarse long-time patterns observed in MO model in contrast with the DA circuits (Fig. 2).

Frequency-selective whitening of Brownian noise

Dimerization process itself generates stochastic fluctuations on a short time scale. However, since this time scale is essentially separated from that of monomer synthesis and decay, dimerization effectively mitigates the monomer-level fluctuations. The frequency content of the fluctuations is best studied by an analysis of the power spectral density (PSD), the original introduced of for signal processing. Fig. 4 shows the noise power spectra of DA1, and the distinction between the MO circuit and the DA topologies is immediately evident. In particular, we note the following two features. (i) A power-law decay with increasing frequency and (ii) a horizontal plateau for the DA circuits. The power-law feature is explained by the "random walk" nature of protein synthesis and decay: The power-law exponent is approximately 2, which is in agreement with a Brownian noise process. Compared to other commonly observed signals, such as white (uncorrelated) noise or $1/f$ noise, protein synthesis/decay has a longer correlation time. If the autocorrelation function of a time course is characterized by a single exponential decay, as is the case for Brownian noise, the Fourier

transform (PSD) is given by Lorentzian profile, thus well approximated by an inverse-square law in the low-frequency regime. We do not observe a saturation value for the MO circuit, and it is likely not in the frequency window of physilogical interest. This may especially be the case when correlation times are long.

The noise reduction is in the physiologically relevant low-frequency regime, and in Fig. 4 we have indicated the typical values for a cell cycle and mRNA half-life. Although stochastic fluctuations impose a fundamental limit in cellular information processing, multiple noise sources may affect cellular physiology non-additively. For a living cell, fluctuations are especially relevant when their correlation time is comparable to, or longer than, the cell cycle. At the same time, short-time scale fluctuations (relative to the cell cycle) are more easily attenuated or do not propagate [24]. Additionally, the observed flat region in the PSD of the DA circuits implies that as far as mid-range frequency fluctuations are concerned, we can safely approximate them as a white noise. This insight may shed light on the reliability of approximation schemes for effetive stochastic dynamcis in protein-only models.

Increased half-life of dimer plays an important role

The virtue of the cytosolic dimer state is also directly related to the extended half-life of proteins when in a complex. Except for the degradation tagging for active proteolysis, a much slower turnover of protein oligomers is the norm. This is partly explained by the common observation that monomers have largely unfolded structures, which are prone to be target of proteolysis [25]. It has also been pointed out that the prolonged half-life of the oligomeric form is a critical factor for enhancing the feasible parameter ranges of gene circuits [26]. As seen from Fig. 3 (also Table), the fold change of the noise reduction, while still significant, is not as strong for the (hypothetical) case of dimer half-life being the same as that of the monomer ($\gamma_2/\gamma_1 = 1/2$). However, the low-frequency power spectra still exhibit almost an order-of-magnitude smaller noise power than in the MO circuit with the same rate parameters (Fig. 4). Hence, the noise reduction capability holds good as as long as the dimer half-life is kept sufficiently long compared with the monomer-dimer transition.

Effects of homo-dimerization in genetic toggle switch

The exceptionally stable lysogeny of the phage λ, for which the spontaneous loss rate is $\lesssim 10^{-7}$ per cell per generation [27, 28], has motivated the synthesis of a genetic toggle switch [14] constructed from a pair of genes, which we will denote genes A and B, that transcriptionally repress each other's expression. This mutual negative regulation, in effect, creates a positive feedback loop for each of the genes and provides the basis for the multiple steady states of the toggle. The existence of multistability, in turn, may be exploited as a device for epigenetic memory or for decision making [?]. As the general attributes of positive feedback suggest, a genetic toggle switch responds to external cues in an ultrasensitive way: When the strength of a signal approaches the threshold value, the gene expression state can be reversed by a small change in the signal. For example, the concentration of protein A (B) may rapidly switch from high (low) to low (high). However, the flip-side of ultrasensitivity is vulnerability to random fluctuations near the threshold.

In a simple model, the monomer-only (MO) toggle, regulatory proteins only exist in monomeric form. Although an external signal is not explicitly included, random fluctuations in the abundance of the circuit's molecular components will occasionally flip the toggle-state for the two protein species. Drawing on the results from our analysis of positive autoregulatory gene circuits, we hypothesize that dimerization in the regulatory proteins of the toggle switch will serve to stabilize its preformance against noise. We allow the protein products of each gene to form a homodimer, being either AA or BB, which is similar to the CI-Cro system in phage λ [29]. We evaluate the effect of the fast protein binding-unbinding dynamics on the toggle switch performance by using either (i) the monomers or (ii) the homodimers as the functional form of the repressor. Figure 5 shows representative time series of the protein monomer (left) and dimer abundances (right) for the case of (a) monomers or (b) dimers as the repressor molecule using several values of the dimer binding affinity K_1.

When the monomer corresponds to the functional form of the repressor molecule (Fig. 5(a)) and K_1 is large (limit of low dimer affinity), the protein populations are dominated by monomers. Hence, the circuit effectively behaves as an MO toggle. As

K_1 decreases, we see that the level of random switching is suppressed: Analogous to the autogenous circuit, the dimer pool stabilizes the protein monomer population. However, the noise suppression is not monotonic with increasing dimer binding affinity. Indeed, for very large binding affinities (small K_1), the number of random switching events is increased since the monomer is only available in low copy numbers. Consequently in this limit, it becomes more likely that a small fluctuation in the monomer abundance can cause a dramatic change in the overall gene expression profile.

The possible noise-stabilizing effect of dimerization is also reflected in the corresponding PSDs (see *Supplementary Information*). We observe a marked suppression of low-frequency fluctuations in the monomer abundance with increasing K_1, as well as a decrease in the high-frequency fluctuations in the dimer abundance with decreasing K_1. This last observation is in contrast with the PSDs for the autogenic circuit (see Fig. 4), where the PSD is mostly independent of the dimer-binding affinity.

In Fig. 5(b) we show corresponding sample time series for the case of a dimeric repressor, all other properties being the same as in (a). While the overall trends are similar, we do note the following difference. Contrary to the monomeric repressor case, there are very few toggle events in the strong binding limit: Since the signaling molecules (dimers) of the dominant gene (the "on"-gene) tend to exist in large copy numbers, a significant fluctation is needed to flip the state of the toggle switch. In the case of monomeric repression, the signaling molecule exists in low abundance in this limit. Thus, the dominant protein species in the dimeric-repressor system is able to maintain much better control over the state of the toggle switch.

In Figure 6, we show the distribution of $(N_A - N_B)$, the difference in molecule abundance for the two protein species in the case of monomeric (left) and dimeric transcription factor (right). The asymmetry with respect to the zero axis is caused by our choice of initial conditions (protein species A in high concentration and species B in low concentration), as well as the finite length of the time series. For monomeric transcription, the presence of dimers with moderate binding affinity sharpens the monomer abundance distribution while accentuating its bimodal character. This is in agreement with the qualitative observation from Figure 5 on switching stability. For dimeric transcription, we clearly observe that the symmetry of the system is broken for small values of K_1, indicating that the state of the toggle switch is extremely stable, and hence, likely determined by the choice of the initial conditions.

To systematically quantify our observations on the interplay between dimer-binding affinity and the functional stability of the toggle switch, we generated long time series ($\approx 3.15 \cdot 10^7$ sec) to measure the average spontaneous switching rate. In Figure 7, we show the average toggle frequency relative to that of the MO toggle for the binding affinities $K_1 = \{2, 20, 100, 1000\}$, and the average MO switching rate is $x \cdot 10^{-6}$. As expected, we find that intermediate values of K_1 are able to stabilize the toggle switch. Figure 7 also highlights the increased stability of the toggle switch for a dimeric versus monomeric transcription factor, the dimeric switching rates always being lower and approaching zero for strong dimer binding.

Heterodimerization in genetic toggle switch

We have also considered the case of heterodimerization in the toggle switch, since the noise- and functional stabilization of the switch may be directly affected by the composition and source of the dimers. Note that, the gene-regulation activity is conferred by the two monomer proteins A and B and not the heterodimer AB. However, we find that the (inactive) heterodimers give rise to very similar noise-stabilizing effects as that of homodimers (Fig. 7). In fact, since the heterodimer state allows the dominant protein species to suppress the (active) monomers of the other protein, the heterodimer toggle switch demonstrates a functional stability similar to the case of homodimeric repressors, and thus, not sharing the discussed vulnerability of monomeric repressor toggle. Although, to our knowledge, this is a purely hypothetical toggle-switch design, it provides a general strategy for noise control in synthetic gene circuits, along with previously proposed approach of overlapping upstream regulatory domains [30].

Conclusions

Cells have evolved distinct strategies to combat the fundamental limits imposed by intrinsic and environmental fluctuations. Recent efforts to correlate network structure with functional aspects may provide valuable insights into approaches for network-

level noise control [31]. While negative feedback is one of the most abundantly observed patterns to achieve the goal of stability, it begs the question fo how cells reliably change the expression of genes from one state to another. The ultrasensitive response circuit, exemplified by the ubiquitous signal transduction cascades in eukaryotic cells, has been proposed as an answer to this question [32, 33].

In addition to the combinatorial expansion of functional specificity, we argue that the availability of oligomeric states contributes to the attenuation of stochastic fluctuations in protein abundace. In positive autoregulatory gene circuits, where the abundance of an expressed protein controls its own synthesis rate, dimerization provides a buffer serving to mitigate random fluctuations associated with the bursty transcription-translation process. We find that, in the frequency domain, short-time binding-unbinding dynamics reduce the overall noise level by converting potentially pathological low-frequency noise to physiologically unimportant, and easily attenuated, high-frequency noise.

Applying this insight to the design of a genetic toggle switch demonstrates the potential use of affinity-manipulation for synthetic biology, where the construction of noise-resistant gene circuits is of eminent importance. In practice, small ligand molecules may be employed to regulate the binding affinity of the regulatory proteins, being either monomers or dimers. Our results further suggest that the structure of the protein-interaction network [?] may provide important insights on methods for genome-level noise control in synthetic and natural systems.

Methods

Model construction

To evaluate the general role of protein oligomerization in a broader functional context, we studied the two most common motifs found in genetic regulatory circuits: positive autoregulation and the bistable switch. We employed the Gillespie algorithm [34] to perform exact stochastic simulations. The reaction scheme studied is summarized in Fig. 1, where the binding/unbinding reactions between RNAp and promoter or between TF and operator are made explicit. Note that, neither binding equilibrium nor empirical Hill-type cooperativity is assumed *ad hoc*. In particular, we split the lumped transcription process into two separate events, (i) isomerization of closed RNAp-promoter complex to its open form and (ii) transcription elongation followed by termination. This is to reflect the availability of the free promoter while the transcription machinery proceeds along the coding sequence of a gene as soon as the promoter region is cleared of the RNAp holoenzyme. Otherwise, the promoter would be inaccessible during a whole transcription event, altering the random mRNA synthesis dynamics.

To realize the genetic switches in a stochastic setting, we keep track of the microscopic origin of cooperativity that gives rise to the multistability by employing (i) multiple operator sites which have the same binding affinity with the repressor, and (ii) formation of dimers (a) between the two repressor proteins (heterodimers), and (b) separately for the repressor proteins (homodimers).

Stochastic simulation

We used the Gillespie direct [34] and Next Reaction (Gibson-Bruck) [35] algorithms, both based on the exact chemical master equation. The Dizzy package [36] were used as the core engine of the simulatons. To ensure that calculations were undertaken in a steady state, we preran a deterministic solver to get the stationary configurations and used them as initial condition of stochastic simulations. For each model system, we generated 10^5 ensemble runs with identical initial conditions and used the instantaneous protein copy number at a fixed time point $t = 5000$ sec. To achieve high-quality power spectra in the low- and high-frequency limits, we ran time courses ($\sim 10^5$ sec) with higher sampling frequency (20 measure points per second).

To calculate the average switching rate, we generated time series of minimum lenght $3.15{\cdot}10^7$ sec (corresponding to 1 year). We identify a state change in the toggle switch by monitoring the ratio of the monomer and dimer abundance for the two protein species. In order to avoid counting short-time fluctuations that do not correspond to a prolonged change of the toggle state, we a applied sliding-window average using a window size of 1000 sec to the time series.

Authors' contributions

CMG and EA designed the study. CMG performed the computations. CMG and EA analyzed the results and wrote the paper. Both authors have read and approved the final version of the paper.

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Figure 1, Ghim & Almaas

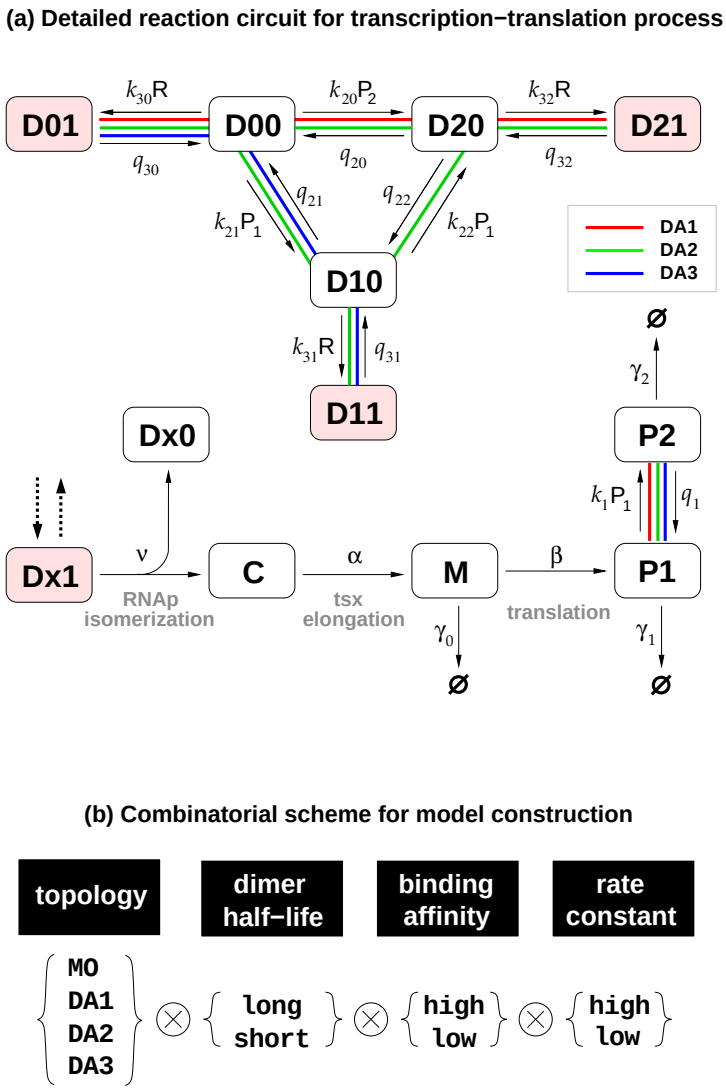

Figure 1: Schematic of model autoregulation gene circuit. (a) The DNA binding status is indicated by Dxy, where x corresponds to the operator region (empty=0, monomer=1, dimer=2), and y to the promoter region (empty=0, RNA polymerase bound=1). C represents the open complex of DNA-RNAp holoenzyme with the promoter sequence just cleared of RNAp and is subject to transcription elongation. Finally, M, P1 and P2 correspond to mRNA, protein monomer, and dimer, respectively. The network topologies can be grouped into two classes, monomer-only (MO) or dimer-allowed (DA) circuits. We have studied DA1 (red lines), which only allows the dimer to bind with the DNA-operator sequence, DA2 (green) with sequential binding of monomers on the DNA, and DA3 (blue), which shares protein-DNA binding kinetics with MO while allowing dimerization in the cytosol. (b) We have investigated the combination of the listed model components assuming cells in the exponential growth phase and the number of RNAp (R) constant.

Figure 2, Ghim & Almaas

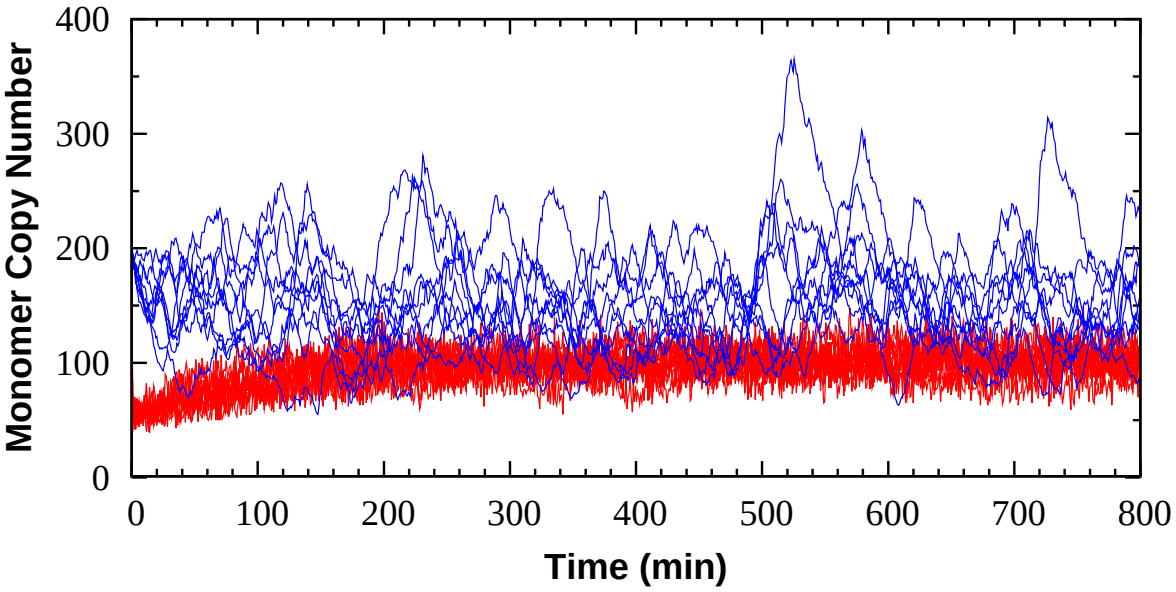

Figure 2: Ten independent time courses of the abundance of protein monomers in the (positive) autoregulatory circuit. The availability of a cytosolic dimer state (red, using circuit topology DA1) significantly reduces the copy-number fluctuations of the monomer compared to the monomer-only (MO) circuit (blue). All corresponding MO and DA1 parameters have the same values.

Figure 3, Ghim & Almaas

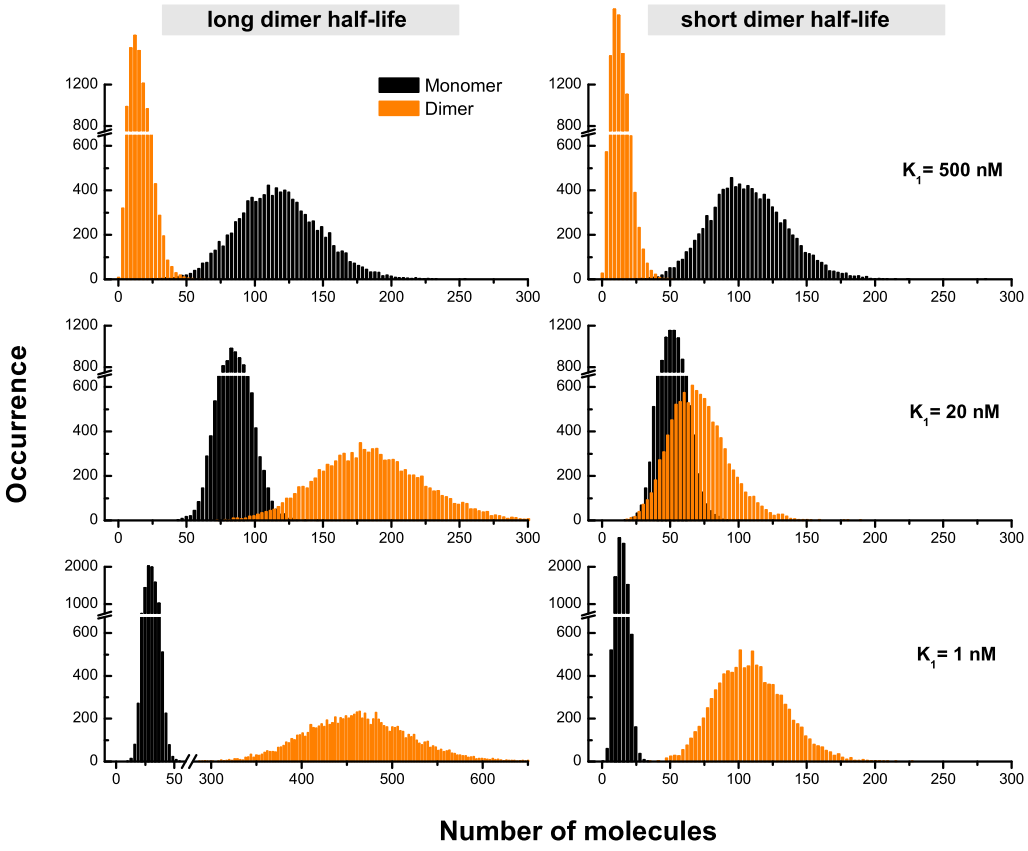

Figure 3: Stationary state distribution of monomer (black) and dimer (orange) protein abundance in the positive autogenous circuit. The left (right) column corresponds to a dimer to monomer dissociation ratio of $\gamma_2/\gamma_1 = 1/10$ ($\gamma_2/\gamma_1 = 1/2$). The molecular copy numbers are collected at a fixed time inverval ($5 \cdot 10^3$ sec) after the steady state has been reached. As the binding equilibrium is shifted towards the dimer state (decreasing K_1), the noise level is monotonically reduced (see Table). Note that the prolonged protein half-life due to the complex formation (left column) affects the noise level.

Figure 4, Ghim & Almaas

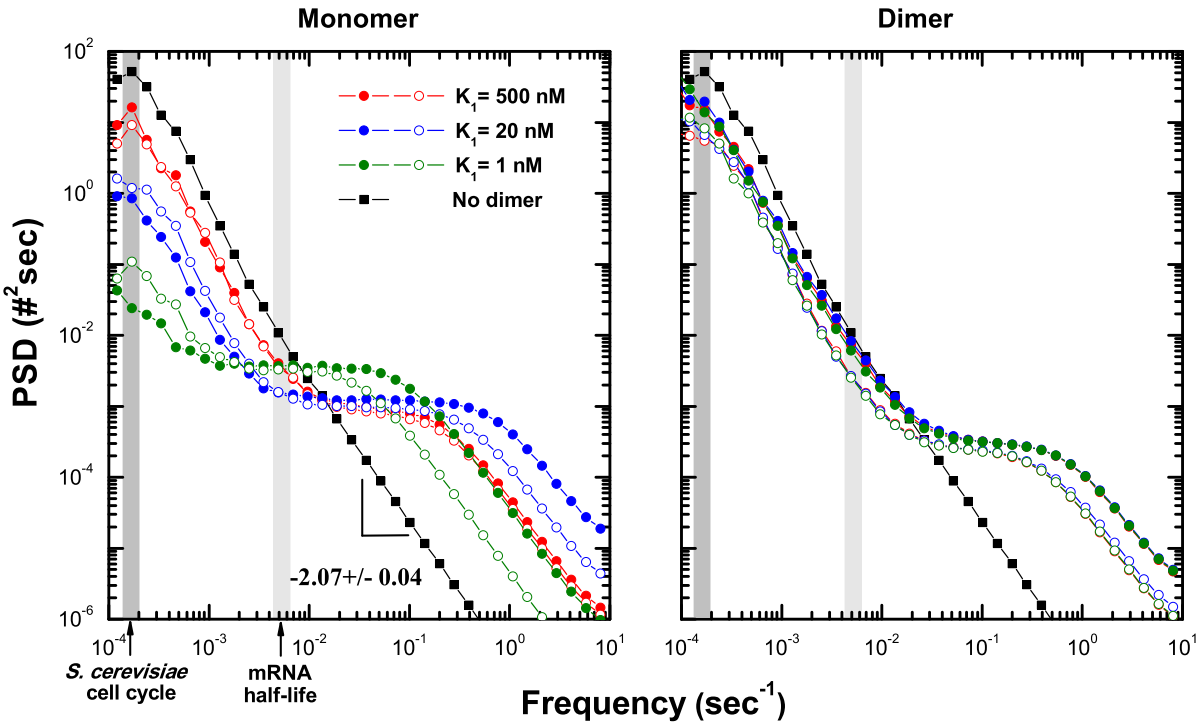

Figure 4: Power spectral density (PSD) of fluctuations in protein abundance. The PSD of the MO circuit clearly displays a power-law behavior. All other model systems with an available cytosolic protein dimer state (DA1 shown here) develop a plateau in the mid-frequency region regardless of the model details (See *Supplementary Information*). As the dimer binding affinity increases, the noise level is further reduced.

Figure 5, Ghim & Almaas

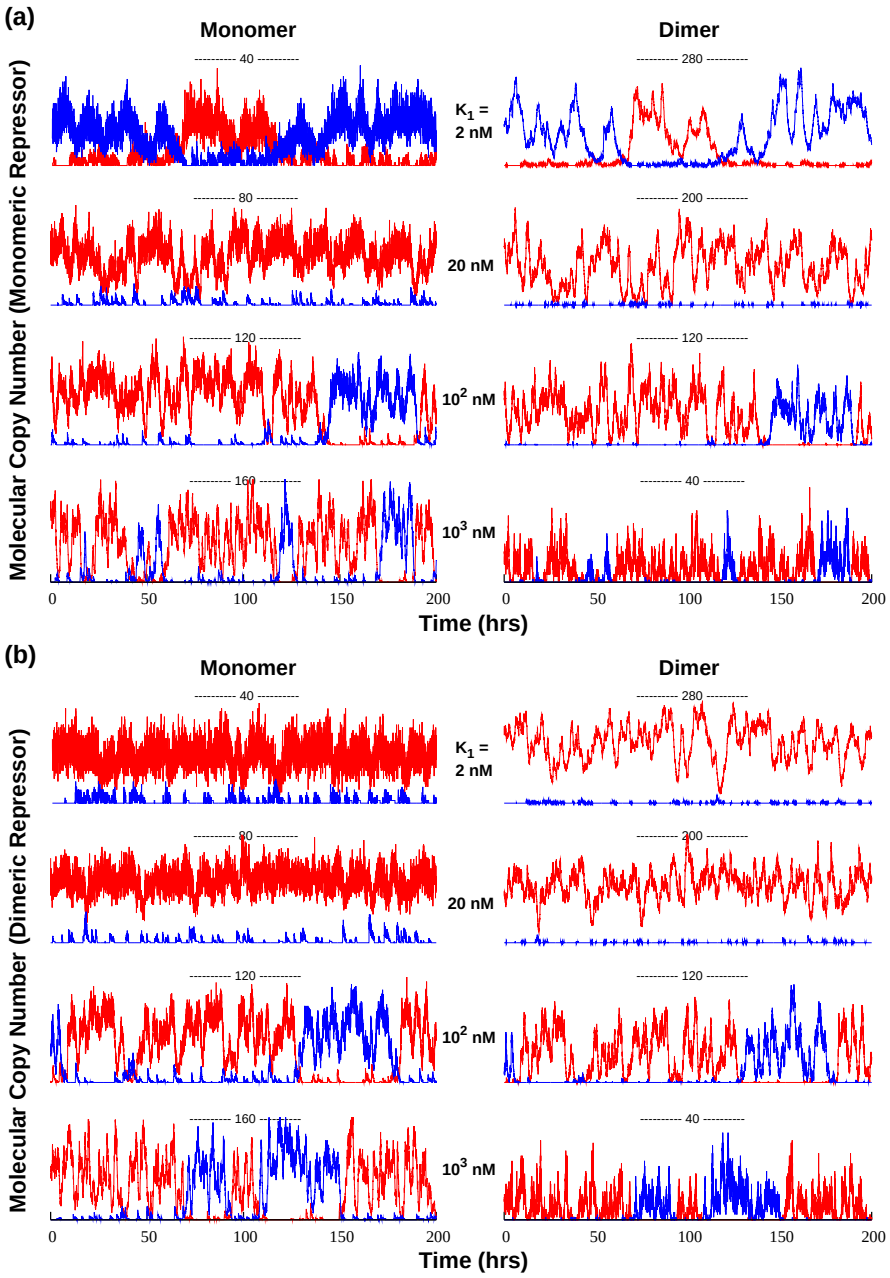

Figure 5: Genetic toggle switch. Time series of monomer and dimer copy numbers after steady state has been reached, where (a) the monomer and (b) the dimer is the functional form of the repressor. The left (right) column corresponds to the number of the two monomer molecules A and B (dimers AA and BB), and the initial state is always with species A (red) in high abundance. Note that the switching frequency depends on the affinity of the dimer state.

Figure 6, Ghim & Almaas

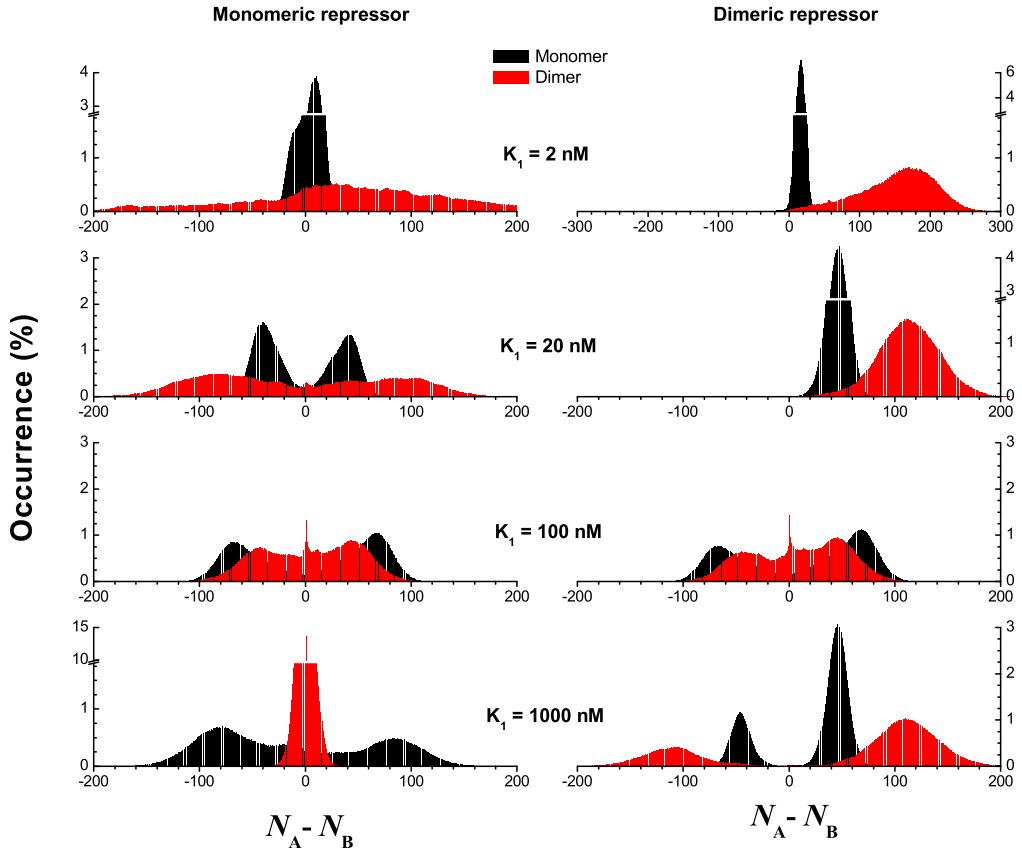

Figure 6: Distribution of monomer abundance differences between protein species A and B. The asymmetry with respect to the zero axis is due to the choice of initial state (species A high) and the finite time span of simulations.

Figure 7, Ghim & Almaas

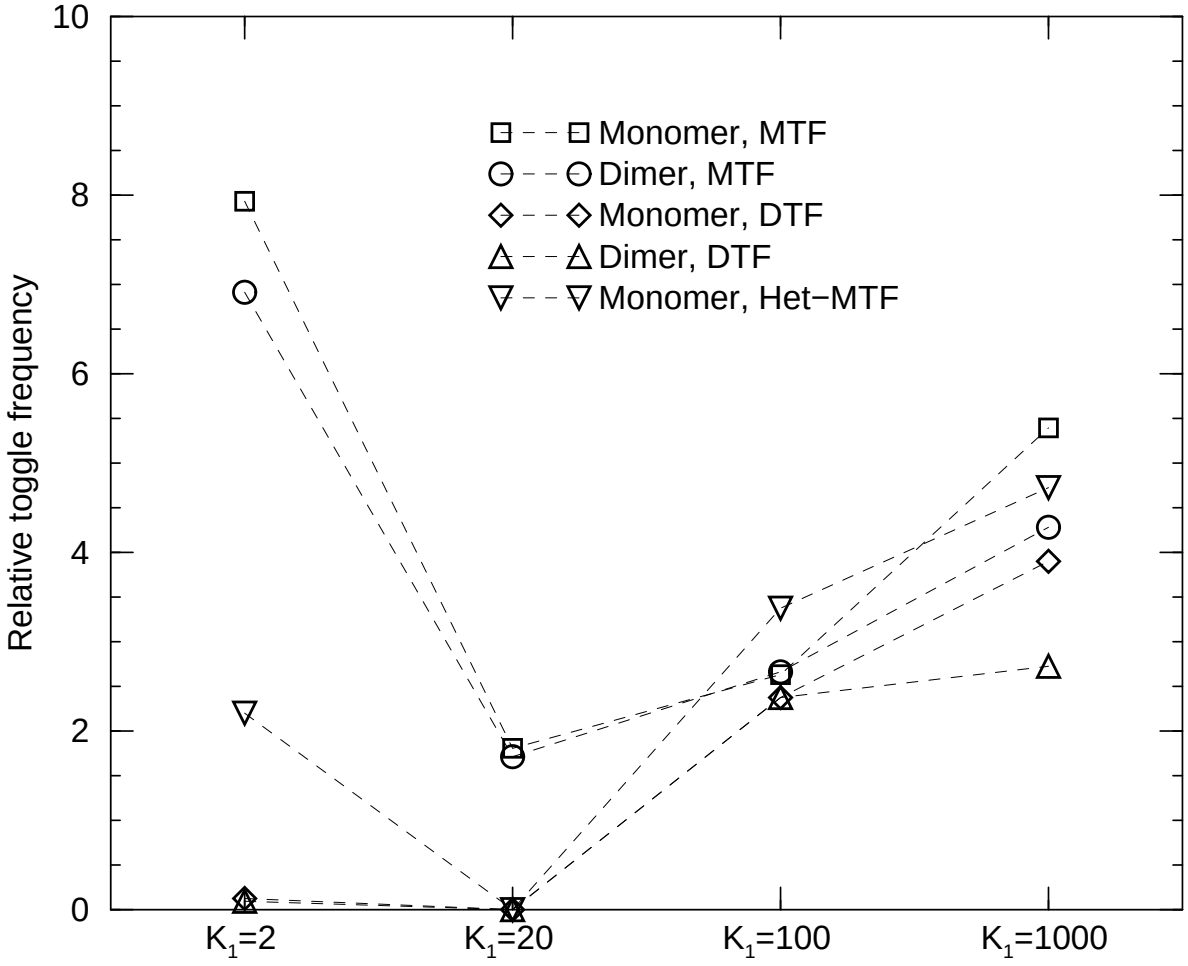

Figure 7: Switching rate of monomers and dimers in a dimeric toggle circuit relative to that of the MO toggle switch. We have investigated monomeric transcription factor (MTF), dimeric transcription factor (DTF), and heterodimers with monomeric transcription factor (Het-MTF) for several values of the dimer binding affinity parameter K_1. The MO switching rate is X.

Tables

Category	Symbol	Reaction	Value (s^{-1})	Ref.
protein dimerization	k_1	$P_1 + P_1 \rightarrow P_2$	0.001-0.1	[37, 38]
	q_1	$P_2 \rightarrow P_1 + P_1$	0.1-1	
TF-operator int	k_{20}	$P_2 + D00 \rightarrow D20$	0.012	[39–41]
	q_{20}	$D20 \rightarrow P_2 + D00$	0.9	
	k_{21}	$P_1 + D00 \rightarrow D10$	0.038	
	q_{21}	$D10 \rightarrow P_1 + D00$	0.3	
	k_{22}	$P_1 + D10 \rightarrow D20$	0.011	
	q_{22}	$D20 \rightarrow P_1 + D10$	0.9	
RNAp-promoter int	k_{30}	$R + D00 \rightarrow D01$	0.038	[42–44]
	q_{30}	$D01 \rightarrow R + D00$	0.3	
	k_{31}	$R + D10 \rightarrow D11$	0.38	
	q_{31}	$D11 \rightarrow R + D10$	0.03	
	k_{32}	$R + D20 \rightarrow D21$	0.38	
	q_{32}	$D21 \rightarrow R + D20$	0.03	
Isomerization	v	$Dx1 \rightarrow C + Dx0$	0.0078	[41]
tsx-tsl elongation & decay	α	$C \rightarrow M + R$	0.03	[45, 46]
	β	$M \rightarrow P_1 + M$	0.044	
	γ_0	$M \rightarrow \varnothing$	0.0039	
	γ_1	$P_1 \rightarrow \varnothing$	7×10^{-4}	
	γ_2	$P_2 \rightarrow \varnothing$	$0.7\text{-}3.5\times10^{-4}$	

Table 1: Kinetic rates for the positive autogenous circuit.

K_1 (nM)	$\gamma_2 = \gamma_1/10$		$\gamma_2 = \gamma_1/2$	
	monomer	dimer	monomer	dimer
500	0.866	0.478	0.826	0.426
20	0.209	0.936	0.230	0.716
1	0.127	0.809	0.132	0.679

Table 2: Relative Fano factors of protein abundance distribution for the autogenous circuit (topology DA1). Reference value for the monomer-only (MO) circuit is 8.729.