Small RNA-driven feed-forward loop: fine-tuning of protein synthesis through sRNA-mediated crosstalk

Swathi Tej$^{1,a}$ and Sutapa Mukherji$^{1,2,b}$

$^1$ Protein Chemistry and Technology, Central Food Technological Research Institute, Mysore, Karnataka 570 020, India
$^2$ Mathematical and Physical Sciences Division, School of Arts and Sciences, Ahmedabad University, Navrangpura, Ahmedabad 380009, India

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Abstract Often in bacterial regulatory networks, small non-coding RNAs (sRNA) interact with several mRNA species. The competition among mRNAs for binding to the common pool of sRNA might lead to crosstalk between the mRNAs. This is similar to the competing endogenous RNA effect that leads to complex gene regulation with stabilized gene expression in Eukaryotes. Here, we study an sRNA-driven feed-forward loop (sFFL) where the top-tier regulator, an sRNA, translationally activates the target protein (TP) as well as a transcriptional activator of the TP through binding to the respective mRNAs. We show that the sRNA-mediated crosstalk between the two mRNA species enables the sFFL to function in three different regimes depending on the synthesis rate of the transcriptional activator mRNA. Of these three regimes, there exists a sensitive regime where the TP level shows interesting features depending on the precise mechanism of target translation. In the case of translation entirely from sRNA–mRNA bound complexes, the TP level becomes maximum around the sensitive regime. Through stochastic analysis and simulations, we show that relative fluctuations in the TP level is minimized here. For translation both from mRNA and sRNA–mRNA bound complexes, the target expression shows a threshold response across the sensitive regime.

1 Introduction

Gene expression is a complex process involving, for example, a number of genes, proteins and regulatory RNAs of different kinds. Different types of molecular mechanism at transcriptional and post-transcriptional levels regulate gene expression process to ensure that the proteins are synthesized to the desired level with the required efficiency. Among various regulatory molecules, small non-coding RNAs (sRNAs) have drawn significant attention in the recent past for their diverse regulatory properties \cite{1,2}. sRNAs are approximately 50–300 nucleotides long and they often regulate the protein synthesis by base-pairing with the target mRNAs leading to mRNA degradation or translational inhibition \cite{2,3}. Although sRNA-mediated interactions are found to be mostly repressing in nature, there are recent reports of activating interactions by sRNAs where sRNAs enhance the stability of mRNAs by sequestering the RNase E recognition site or facilitate translation initiation by opening the sequestered ribosome binding site of the secondary structure mRNAs \cite{3,4}. Since sRNAs do not code for proteins, it is generally believed that sRNAs lead to fast and energy-efficient gene regulation.

A number of interesting properties are found in the case of sRNA-mediated gene regulation. For instance, in the case of only sRNA-mediated regulation, the target protein concentration shows a threshold linear response \cite{5}. In the case of combined transcriptional and translational regulation by proteins and sRNAs, respectively, one, however, finds both monostable (with threshold linear response) and bistable regions in the target protein concentration \cite{6}.

Gene expression is inherently stochastic due to the probabilistic nature of various molecular interactions associated with gene expression \cite{7,8}. The stochasticity leads to random fluctuations in the protein levels although it is known that many biological functions of the cell require fine-tuning of necessary protein levels. In this regard, it has been found that sRNA-mediated repression of gene expression leads to reduced fluctuations in the protein level as compared to transcription factor-mediated repression \cite{5}. Further, optimal noise buffering has also been seen in more complex genetic circuits such as incoherent sRNA-mediated feed-forward loops (FFL) \cite{9,10} and sRNA-driven feed-forward loop \cite{11}, where dual strategies i.e., regulation at both transcriptional and translational levels are employed.
Recent studies have revealed a particularly subtle and complex strategy of miRNA-mediated gene regulation, wherein a given species of miRNA interacts with a number of different mRNA targets [12–16]. The primary goal of such miRNA–mRNA network is to give rise to regulation through competition in which different mRNA targets compete for binding to the same miRNA species (also known as competing endogenous RNA or ceRNA effect). In other words, the miRNAs may function as a channel through which the change in concentration level in one type of target mRNA can be conveyed to another. It has been shown that such indirect miRNA-mediated crosstalk may outperform direct regulation under certain circumstances [15]. Additionally, it has been found that such miRNA-mediated crosstalk between mRNAs results in a broad impact on the protein level such as enhancing the stability of highly expressed proteins, altering the correlation patterns of co-regulated interacting proteins apart from, in general, fine-tuning the protein levels [16].

In the present paper, our attention is on a network motif where the sRNA-mediated crosstalk between mRNAs seems to play an important role in regulating the target protein synthesis. Network motifs are specific sub-networks that have frequent recurrences in large regulatory networks as some of their major building blocks [17]. Such motifs usually have distinct functionalities and it is believed that such motifs are chosen evolutionarily due to distinct advantages they provide to the cell. The network motif of our interest is a feed-forward loop (FFL) which is driven by an sRNA (see Fig. 1a) unlike the commonly found FFLs driven by transcription factors [18].

We refer to this sRNA-driven FFL as sFFL in the following. In general, the top-tier regulator in FFL drives the target protein synthesis along two pathways; one pathway involves a direct regulation of the target protein synthesis and the other involves an indirect regulation via up- or down-regulation of an intermediate regulator of the target protein. For a purely transcriptional FFL, all the interactions are at the transcription level and the top-tier as well as the intermediate regulators are transcription factors. The sFFL introduced here is different from another kind of transcription factor driven FFLs which involve sRNA as an intermediate regulator of the target protein [10,19,20]. The feature that remains common in all these FFLs is that the top-tier regulator regulates expression of two distinct genes. In the case of sFFL, an sRNA being the top-tier regulator regulates the translational activities of two different mRNAs along two regulatory pathways of FFL and thus gives rise to distinct regulatory features through sRNA-induced crosstalk between mRNAs.

The existence of such sFFL has been found experimentally very recently in the context of stress response of *Salmonella enterica* subjected to stress due to a bactericidal agent, bile salt [18]. In this sFFL, an sRNA, RprA, activates the synthesis of the target protein, RicI, directly through translational activation of RicI mRNA and indirectly via translational activation in the synthesis of the alternative sigma-factor, $\sigma^s$, which transcriptionally activates RicI gene (see Fig. 1a and b). By base-pairing with the $\sigma^s$ mRNA, RprA opens up the translation inhibitory loop in the 5′ untranslated region (UTR) of $\sigma^s$-mRNA and facilitates ribosome binding to initiate translation leading to the synthesis of $\sigma^s$ protein [21]. $\sigma^s$ protein being a transcriptional activator of RicI gene leads to an enhanced synthesis of RicI-mRNA which are then translationally activated by sRNA, RprA, resulting in an up-regulation in RicI protein synthesis. Here again, RprA facilitates ribosome binding by opening-up the translation inhibitory loop of RicI-mRNA. An up-regulation of RicI protein happens in response to the membrane damaging activity of the bile salt. As a response to the stress, the bacterial cell prefers to shut down the energy-expensive processes associated with horizontal gene transfer although horizontal gene transfer plays a significant role in bacte-

![Fig. 1](https://example.com/fig1.png) **Fig. 1** (a) An sRNA-driven feed-forward loop (sFFL) found in the *Salmonella enterica*. The top-tier regulator is an sRNA, RprA, which leads to translational up-regulation of two proteins i.e., $\sigma^s$ and RicI. (b) A diagram with the details of all the processes such as synthesis, degradation of all the components as well as the complex formation between sRNA and mRNAs. $c_1$ and $c_2$ denote the complexes RprA-$\sigma^s$ mRNA and RprA-RicI mRNA, respectively.
ria’s survival under normal conditions. By interfering with the formation of the pilus that is necessary for bacterial conjugation during horizontal gene transfer, RicI protein down-regulates the process of horizontal gene transfer and protects the bacteria from additional energy loss associated with this process [18]. For the rest of the paper, we follow general notations for various regulatory molecules as listed in Table 1.

Although miRNA-mediated crosstalk between different mRNAs has been a subject of extensive investigations recently, the present work is different from earlier studies in several respects. Instead of sRNA-mediated repression, which is the most common form of regulation by sRNAs, here the sRNA leads to translational activation of two different mRNAs. Further, the sFFL not only involves sRNA-mediated interactions between the mRNAs, but it also involves an interaction between the mRNAs mediated by $p_1$. The latter is due to the fact that an up-regulation of one species of mRNA ($m_1$ i.e., $\sigma^s$ mRNA) gives rise to enhanced transcription of the other species of mRNA ($m_2$ i.e., RicI mRNA) via up-regulation of the protein product of $m_1$, ($p_1$ i.e., $\sigma^s$ protein) [18]. Overall, in contrast to the common scenario where sRNA-mediated interaction establishes links between different sub-networks, here the sRNA-mediated crosstalk influences the target protein synthesis within a single network motif.

Using various tools of mathematical modelling, we quantify the effect of sRNA-mediated crosstalk on the target protein concentration. The crosstalk is seen by studying how the concentration of mRNA2 ($m_2$) and sRNA ($s$) change as the synthesis rate of mRNA1 ($m_1$) is changed. Following the table, we refer the mRNAs as well as their concentrations as $m_1$ and $m_2$. The same convention is followed for other regulatory components as well. As the concentration of $m_1$ increases, there is a reduction in the free sRNA concentration since $m_1$ molecules tend to form complexes with available free sRNAs and this, consequently, leads to an increase in the concentration of free $m_2$ molecules since sRNAs are largely bound to $m_1$. Interestingly, the change in the concentrations shows a sensitive (or susceptible) region over a narrow range of $m_1$ synthesis rate ($r_{m_1}$), where with a small increase in $r_{m_1}$, there is an abrupt change in various concentrations. We find that the change in the target protein level with $r_{m_1}$ strongly depends on the precise mechanism of target protein translation. Two possibilities are considered here. (1) In the case of target translation from sRNA–mRNA bound complexes, the sRNA-mediated crosstalk leads to an initial increase in the target protein concentration with $r_{m_1}$. With further increase in $r_{m_1}$, the target protein concentration reaches a peak and then undergoes a sharp decrease. This maximum and the subsequent sharp decrease in the target protein concentration with $r_{m_1}$ appear over the sensitive region. Further, over this sensitive region, the relative fluctuation in the target protein level becomes the least. (2) In the case of target protein translation from mRNA (without the involvement of sRNA) as well as from sRNA–mRNA bound complexes, the target protein concentration shows a threshold response with $r_{m_1}$. The target protein concentration remains low initially for low $r_{m_1}$, and increases significantly beyond a threshold value of $r_{m_1}$, with the threshold region coinciding with the sensitive region. This is in sharp contrast to an sRNA-driven cascade network (sCN) where the sRNA–mRNA2 complex formation is absent, and the target translation takes place from the target mRNA without the involvement of sRNA (see Fig. 7b in “Appendix A1”). In the case of sCN, the target protein concentration increases smoothly as $r_{m_1}$ increases.

### Table 1 Mathematical notations for concentrations of various components of sFFL

| Notations    | Biological sFFL                                      |
|--------------|------------------------------------------------------|
| $s$ (sRNA)   | RprA sRNA                                            |
| $m_1$ (mRNA1)| $\sigma^s$ mRNA                                      |
| $c_1$ (complex-1) | RprA-$\sigma^s$mRNA complex                          |
| $p_1$ (protein-1) | $\sigma^s$ protein                                   |
| $m_2$ (mRNA2) | RicI mRNA                                            |
| $c_2$ (complex-2) | RprA-RicI mRNA complex                               |
| $p_2$ (protein-2) | RicI protein                                         |

2 Results

2.1 Model and steady-state results

In the following, we present a model for the sFFL describing how the concentrations of various regulatory components change with time. The equations that we use for our calculations are

\[
\dot{s} = r_s - \gamma_s s - k_1^+ s m_1 - k_2^- s m_2 + (k_1^- + \kappa_1)c_1 + (k_2^- + \kappa_2)c_2, \tag{1}
\]

\[
\dot{m}_1 = r_{m_1} - \gamma_{m_1} m_1 - k_{1}' s m_1 + k_{1}' c_1, \tag{2}
\]

\[
\dot{c}_1 = k_1^+ s m_1 - (k_1^- + \gamma_1 + \kappa_1)c_1, \tag{3}
\]

\[
\dot{p}_1 = r_{p_1} c_1 - \gamma_{p_1} p_1, \tag{4}
\]

\[
\dot{m}_2 = \frac{r_{m_2} k_{c_1} p_1}{1 + k_{c_1} p_1} - \gamma_{m_2} m_2 - k_{1}^+ s m_2 + k_{2}^- c_2, \tag{5}
\]

\[
\dot{c}_2 = k_2^+ s m_2 - (k_{2}^- + \gamma_2 + \kappa_2)c_2, \tag{6}
\]

\[
\dot{p}_2 = r_{p_2} c_2 - \gamma_{p_2} p_2, \tag{7}
\]

where in general, $\dot{x} = \frac{dx}{dt}$. Here, we have used general notations for various concentrations as given in Table 1. The rate parameters $r$ and $\gamma$ represent synthesis and degradation rates in general, $c_1$ and $c_2$ represent sRNA–mRNA complexes of two different kinds. $k_1^+$ and $k_1^-$ represent the association and dissociation rates of these complexes. The degradation pathways of the sRNA–mRNA complex can be of two different kinds. While in the stoichiometric pathway, the sRNA is degraded along with the mRNA, in the case of the catalytic path-
way, mRNAs are degraded while sRNAs are recycled back into the system [22]. Although at present, the primary reason behind a specific sRNA–mRNA complex degradation pathway is unclear, it is believed that the structure of the sRNA–mRNA bound complex and the details of the cleavage mechanism play an important role in deciding the degradation pathway. The equations above take into account both catalytic and stoichiometric pathways with the rate constants 

\[ \kappa_i \] and \( \gamma_i \) refer to the rates associated with complex \( c_i \).

In the steady state, the solutions for \( m_1 \) and \( m_2 \) are

\[
m_1 = m^*_1 F_1(s), \quad m_2 = \frac{m^*_2 k_c a s F_1(s) F_2(s)}{1 + k_c a s F_1(s)},
\]

where

\[
F_1(s) = \frac{1}{1 + s/s_{01}} \text{ with } s_{01} = \frac{\gamma_{m_1} k_{i_1}^+ + \gamma_1 + \kappa_1}{k_1^+ \gamma_1 + \kappa_1},
\]

\[
F_2(s) = \frac{1}{1 + s/s_{02}} \text{ with } s_{02} = \frac{\gamma_{m_2} k_{i_2}^+ + \gamma_2 + \kappa_2}{k_2^+ \gamma_2 + \kappa_2},
\]

\[
\frac{r_{m_1}}{\gamma_{m_1}}, \quad \frac{r_{m_2}}{\gamma_{m_2}}, \quad a = \frac{r_{p_1}}{\gamma_{p_1}} \frac{k_1^+ m_1^*}{k_1^+ + \gamma_1 + \kappa_1}.
\]

The steady-state concentration of sRNA can be found upon solving the following algebraic equation for \( s \)

\[
r_s - \gamma_s s - r_{m_1} \zeta_1 s F_1(s) - r_{m_2} \zeta_2 s \frac{k_c a s^2 F_1(s) F_2(s)}{1 + k_c a s F_1(s)} = 0,
\]

with \( \zeta_1 = \frac{k_{i_1}^+ \gamma_1}{\gamma_{m_1} (k_1^+ + \gamma_1 + \kappa_1)}, \quad \zeta_2 = \frac{k_{i_2}^+ \gamma_2}{\gamma_{m_2} (k_2^+ + \gamma_2 + \kappa_2)} \).

Figure 2 shows how sRNA and mRNA concentrations change as \( r_{m_1} \), the transcription rate of \( m_1 \), is changed.

The presence of sRNA-induced effective interaction between \( m_1 \) and \( m_2 \) is apparent here since a change in the transcription rate of \( m_1 \) affects the equilibrium concentrations of the sRNA and \( m_2 \). Intuitively, as the concentration of \( m_1 \) increases, these mRNAs bind the sRNAs causing a reduction in the concentration of free sRNAs and, as a consequence, an increase in the concentration of free \( m_2 \). Interestingly, Fig. 2 shows that over a narrow range of \( r_{m_1} \), there exists a sensitive region where the concentrations of \( s, m_1 \) and \( m_2 \) change sharply as \( r_{m_1} \) is changed. We shall show in the following that the target protein concentration reaches its maximum value in this region along with least relative fluctuation around the average concentration. Since the protein product of \( m_1 \) (i.e., \( p_1 \)) is a transcriptional activator of \( p_2 \), the change in the concentration of \( m_2 \) with \( r_{m_1} \) is expected. However, as we show below, a large contribution to the increase in \( m_2 \) concentration comes from the sRNA-induced crosstalk between mRNAs.

### 2.2 Response functions

In order to quantify the interactions among various regulatory molecules, we introduce the following response functions:

\[
\chi_{ij} = \frac{\partial m_i}{\partial r_{m_j}}, \quad \text{where } i, j = 1, 2 \text{ with } i \neq j \quad (14)
\]

\[
\chi_{si} = \frac{\partial s}{\partial r_{m_i}}, \quad \text{where } i = 1, 2. \quad (15)
\]

Here, \( \chi_{ij} \) represents the response in terms of a change in the concentration of a specific kind of mRNA (\( m_1 \) or \( m_2 \)) as the transcription rate of the other mRNA is changed. Similarly, \( \chi_{si} \) represents the change in sRNA concentration as the transcription rates of the \( i \)th mRNA, i.e., \( r_{m_1} \) or \( r_{m_2} \) is changed. Using Eqs. (8) and (12), we find the response functions as

\[
\chi_{ij} = \frac{\partial m_i}{\partial r_{m_j}}, \quad \text{where } i, j = 1, 2 \text{ with } i \neq j \quad (14)
\]

\[
\chi_{si} = \frac{\partial s}{\partial r_{m_i}}, \quad \text{where } i = 1, 2. \quad (15)
\]
The vanishing of $\chi_{12}$ is expected as, in contrary to sFFL, here the increase in $m_2$ synthesis does not affect the sRNA concentration.

In order to see how the response function $\chi_{21}$ changes with $r_{m_1}$, we have obtained $\chi_{21}$ numerically using Eqs. (8) and (12). For an estimate of the contribution coming from the direct interaction between $m_1$ and $m_2$ (mediated via the transcriptional activator $p_1$), we have plotted the second term of $\chi_{21}$ in Eq. (16), $\chi_{21a} = \frac{m_2 k_c a s F_1}{r_{m_1} (1+k_c a s F_1)}$ after substituting the solution for $s$ as a function of $r_{m_1}$. Figure 3a shows the rate of change of concentration of $m_2$ with respect to $r_{m_1}$ (i.e., $\chi_{21}$) as a function of $r_{m_1}$. The presence of the sensitive region, as discussed before, appears very prominently in these figures. As Fig. 3a and b indicates, the contribution of the interaction between mRNAs (via the transcriptional activator, $p_1$) to $\chi_{21}$, in general, is quite small compared to the direct interaction term.
to the sRNA-mediated part. The response function for protein is discussed in “Appendix A2”.

### 2.3 Effect of sRNA-mediated crosstalk on the target protein concentration

In this section, we focus on the influence of sRNA-mediated crosstalk between mRNAs on the target protein concentration. In the case of sRNA recycling only (i.e., $\gamma_1 = \gamma_2 = 0$ and $\kappa_1, \kappa_2 \neq 0$), there is no sRNA-mediated crosstalk between the mRNAs since $\chi_s = \chi_{s2} = 0$. Under such conditions, the target protein concentration increases initially with the transcription rate, $r_{m1}$, and saturates eventually as a consequence of the saturation kinetics associated with transcriptional activation in $m_2$ synthesis by $p_1$ (see Fig. 4a). In the presence of sRNA-induced crosstalk (i.e., $\gamma_1, \gamma_2 \neq 0$), with the increase in $r_{m1}$, the target protein concentration reaches a peak followed by a sharp decrease. This happens near the sensitive region where the concentration of free sRNA available for translational activation of $p_2$ drops down drastically. In order to have a meaningful comparison with the sCN, we include in sFFL also the possibility of target protein synthesis, at rate $r_{P2}$, from $m_2$ without sRNA facilitating its translation. This is considered in addition to target protein synthesis from the sRNA-mRNA2 bound complex (i.e., $c_2$). Figure 4b shows an interesting difference in the target protein levels of sFFL and sCN. While sCN shows a smooth increase in the target protein concentration with $r_{m1}$ followed by a saturation, in the case of sFFL, the target protein level shows a threshold-type response with an abrupt increase from a low value near the sensitive region. It appears that for sFFL, for low $r_{m1}$, sRNA binds to both the mRNA species and the target protein synthesis largely happens from the bound complexes. The sharing of sRNA by two species of mRNA leads to reduced synthesis of $p_1$ and hence less transcriptional activation in $p_2$ synthesis by $p_1$ as compared to sCN. For large $r_{m1}$, sRNAs preferentially bind to $m_1$, leading to a large synthesis of $m_2$ and consequently $p_2$ via direct translation at a rate $r_{P2}$. Further, the effect of crosstalk is significantly sensitive to $r_{P2}$, and as shown in “Appendix A3”, the dependence of the target protein concentration on $r_{m1}$ changes significantly even when $p_2$ is synthesized at a basal rate (low values of $r_{P2}$). Further, as expected, due to direct translation at a basal rate, the protein concentration does not drop down as shown in Fig. 4a. These variations in the target protein level indicate that crosstalk leads to a strong $m_1$ ($\sigma^+)$-dependent target protein regulation in sFFL.

In “Appendix A4”, we show how the target protein concentration depends on other parameters, including the synthesis rate of $m_2$ and various degradation rates. These figures indicate that while there are quantitative variations in the target protein concentration depending on the parameter values, the qualitative features of the crosstalk driven regulation are somewhat robust.

![Fig. 4](image-url)

**Fig. 4** Variation of target protein expression with the transcription rate ($r_{m1}$) of mRNA, $m_1$, a sFFL with crosstalk ($\gamma_1, \gamma_2 \neq 0$) and without crosstalk ($\gamma_1 = \gamma_2 = 0$), b sFFL with additional direct synthesis of $p_2$ from $m_2$ at a rate $r_{P2} = 0.01$ and sCN. The insets show the regions marked by the rectangles. Parameter values are the same as those of Fig. 2

### 2.4 Noise analysis

In the previous sections, we were primarily concerned with variations in the average concentrations of different regulatory molecules with specific parameters. The probabilistic nature of biochemical reactions, however, leads to fluctuations in the number of the regulatory molecules about the average values. The aim of the present section is to employ a stochastic analysis that allows us to quantify fluctuations in various molecules. In particular, we are interested in finding out if the sRNA-induced crosstalk affects the fluctuations in the target protein level in a specific manner. For the following analysis, we consider $r_{P2} = 0$ i.e., the target protein synthesis happens only from the bound complex of sRNA and mRNA2.

We begin with the master equation that describes how the probability of a given state changes with time [23]. A state of this system is described by the numbers of sRNA, two different mRNAs and two different protein molecules. The state of the system changes as the numbers of various molecules change due to possible reactions taking place at given rates. We introduce $P_{s,m_1,p_1,m_2,p_2}(t)$ as the probability at time $t$ that
the system is in a state in which the number of sRNA, mRNA and protein molecules are $s$, $m_1$, $m_2$, $p_1$ and $p_2$, respectively. The master equation describing the rate of change of the probability function with time can be written as

$$\partial_t P_{s,m_1,p_1,m_2,p_2}(t) = r_s (P_{s-1,m_1,p_2,m_2} - P_{s,m_1,p_2,m_2}) + \gamma_s ((s+1)P_{s+1,m_1,p_1,m_2,m_2} - sP_{s,m_1,p_1,m_2,m_2}) + r_{m_1} (P_{s,m_1-1,p_1,m_2,p_2} - P_{s,m_1,p_1,m_2,p_2}) + \gamma_{m_1} ((m_1+1)P_{s,m_1+1,p_1,m_2,p_2} - m_1P_{s,m_1,p_1,m_2,p_2}) + r_{m_2} (P_{s,m_1,p_1-1,m_2,p_2} - P_{s,m_1,p_1,m_2-1,p_2}) + \gamma_{m_2} ((m_2+1)P_{s,m_1,p_1,m_2+1,p_2} - m_2P_{s,m_1,p_1,m_2,p_2}) + r_{p_1} s m_1 (P_{s,m_1-1,p_1,m_2,p_2} - P_{s,m_1,p_1,m_2,p_2}) + \gamma_{p_1} ((p_1+1)P_{s,m_1+1,p_1,m_2,p_2} - p_1P_{s,m_1,p_1,m_2,p_2}) - s m_2 (P_{s,m_1+1,p_1,m_2,p_2} - P_{s,m_1,p_1,m_2,p_2}) + r_{p_2} s m_2 (P_{s,m_1,p_1,m_2-1} - P_{s,m_1,p_1,m_2,p_2}) + \gamma_{p_2} ((p_2+1)P_{s,m_1,p_1,m_2+1} - p_2P_{s,m_1,p_1,m_2,p_2}) - s m_2 (P_{s,m_1+1,p_1,m_2,p_2} - P_{s,m_1,p_1,m_2,p_2})$$

(21)

Next, we consider the moment generating function

$$G(z_1, z_2, z_3, z_4, z_5) = \sum_{s,m_1,p_1,m_2,p_2} z_1^s z_2^{m_1} z_3^{p_1} z_4^{m_2} z_5^{p_2} P_{s,m_1,p_1,m_2,p_2}$$

(24)

Here, $\partial_t P_{s,m_1,p_1,m_2,p_2}(t) = \frac{\partial}{\partial t} P_{s,m_1,p_1,m_2,p_2}(t)$. This equation is based on a quasi-steady-state approximation, wherein we assume that the process of complex formation attains steady state faster as compared to other processes such as translation or transcription. This assumption simplifies the mathematical analysis significantly, and we show that the mathematical results agree reasonably well with simulations which do not involve this approximation. Various terms on the right-hand side of the equation account for different reactions representing the synthesis and degradation of various molecules (see “Appendix B1” for details). Here,

$$r_{p_1} = \frac{r_{p_1} k_1^+}{k_1^+ + \gamma_1 + \kappa_1}, \quad r_{p_2} = \frac{r_{p_2} k_2^+}{k_2^+ + \gamma_2 + \kappa_2},$$

$$g_1 = \frac{k_1^+ \gamma_1}{k_1^+ + \gamma_1 + \kappa_1}, \quad g_2 = \frac{k_2^+ \gamma_2}{k_2^+ + \gamma_2 + \kappa_2},$$

$$d_1 = \frac{k_1^+ \kappa_1}{k_1^+ + \gamma_1 + \kappa_1} \quad \text{and} \quad d_2 = \frac{k_2^+ \kappa_2}{k_2^+ + \gamma_2 + \kappa_2}.$$  

(22)

The transcriptional activation of $p_2$ by $p_1$ is taken into account by the Hill function, $r_{m_2}(p_1) = \frac{r_{m_2} k_{p_1}}{1 + k_c (p_1)}$. For the present calculation, the Hill function is approximated about the average steady-state density $(\langle p_1 \rangle)$ as

$$r_{m_2}(p_1) = r_{m_2}^0 + r_{m_2}^1 \langle p_1 \rangle,$$

(23)

where

$$r_{m_2}^0 = \frac{r_{m_2} k_c^2 \langle p_1 \rangle^2}{(1 + k_c \langle p_1 \rangle)^2} \quad \text{and} \quad r_{m_2}^1 = \frac{r_{m_2} k_c^2}{(1 + k_c \langle p_1 \rangle)^2}.$$

Here, $\partial_t G = r_s (z_1 - 1) G + \gamma_s (1 - z_1 - 1) \partial_{z_1} G + r_{m_1} (z_2 - 1) G + \gamma_{m_1} (1 - z_2 - 1) \partial_{z_2} G + r_{p_1} s z_1 z_2 (z_3 - 1) \partial_{z_1 z_2} G + \gamma_{p_1} (1 - z_3 - 1) \partial_{z_3} G + r_{p_2} s z_1 z_2 (z_4 - 1) \partial_{z_1 z_2} G + \gamma_{p_2} (1 - z_4 - 1) \partial_{z_4} G +$$

$$+ r_{m_2} z_3 (z_4 - 1) \partial_{z_3} G + \gamma_{m_2} (1 - z_4 - 1) \partial_{z_4} G + r_{p_2} s z_1 z_2 (z_5 - 1) \partial_{z_1 z_2} G + \gamma_{p_2} (1 - z_5 - 1) \partial_{z_5} G + g_1 (1 - z_2 \langle z_1 \rangle^2 + 2 s (1 - z_3) \partial_{z_1 z_2} G + G_2 (1 - z_4) \partial_{z_1 z_2} G +$$

$$+ d_1 \langle 1, 2 \rangle \partial_{z_1 z_2} G + d_2 \langle 1, 4 \rangle \partial_{z_1 z_2} G.$$

(25)

where $\partial_{z_i} G = \frac{\partial}{\partial z_i} G$ and $\partial_{z_1 z_2} G = \frac{\partial^2}{\partial z_1 \partial z_2} G$.

In the steady state ($\partial_t G = 0$), the right-hand side of Eq. (25) is equated to zero. Various average quantities (moments) can be calculated by differentiating the resulting steady-state equation with respect to appropriate $z_i$ and then considering $z_i = 1$ for all $i$. The first moments follow from $G$ as $G_1 = \langle s \rangle$, $G_2 = \langle m_1 \rangle$, $G_3 = \langle p_1 \rangle$, etc., where $G_i = \partial_{z_i} G |_{\{z_i\}=1}$ with $\{z_i\} = 1$ indicating $z_i = 1$ for all $i$. The second moments similarly can be determined from $G_{ij}$ where $G_{ij} = \partial_{z_i z_j} G |_{\{z_i\}=1}$. We are in particular interested in the relative fluctuation in the target protein level which can be measured through the coefficient of variation, $CV_{p_2} = \langle (p_2 - \langle p_2 \rangle)^2 \rangle^{1/2} / \langle G_{55} + G_5 - G_2^2 \rangle^{1/2} / G_5$. The derivation of the moments $G_5$ and $G_{55}$ appears to be complex since the evaluation of a moment of a given order involves evaluation of various higher-order moments. In order to simplify the derivation, we restrict ourselves up to second moments and express the third
coefficients of variation plotted with for this are Mathematica (lines without markers). The parameter values used for this are \( \gamma_s = \gamma_{m_1} = \gamma_{m_2} = \gamma_{p_1} = \gamma_{p_2} = 0.001, \)
\( r_{m_2} = r_{p_1} = r_{p_2} = 0.01, \) \( k_1^+ = k_2^+ = 0.1, \) \( k_1^- = k_2^- = 0.05, \)
\( k_c = 0.1 \) and \( \gamma_1 = \gamma_2 = 0.005. \) The lines with markers represent the same found from stochastic simulations based on the Gillespie algorithm. The details regarding the reactions and the reaction rates are presented in “Appendix D”.

Moments required for the evaluation of the coefficient of variation are found numerically using Mathematica. The coefficient of variation as a function of \( r_{m_1} \) for different values of \( r_s \), the sRNA synthesis rate, is shown in Fig. 5. It is clear that the relative fluctuation in the target protein concentration is minimum over a range of \( r_{m_1} \). Interestingly, this minimum region overlaps with the sensitive region shown in Fig. 2. As \( r_s \) increases, the sensitive region extends towards larger \( r_{m_1} \). Figure 5 shows that the minimum fluctuation region also changes accordingly, and there is a systematic increase in the fluctuation beyond this region. In order to see the role of crosstalk in noise processing characteristics, we have also obtained the coefficient of variation for the target protein number for sFFL with \( \gamma_1 = \gamma_2 = 0 \) (no crosstalk) and sCN (see “Appendices B2 and C”). The coefficients of variation plotted with \( r_{m_1} \) indicate the absence of an optimum in noise attenuation (see Figs. 12 and 13 in the “Appendix”).

In order to verify the above observations, we obtain the coefficient of variation of the target protein number from stochastic simulations based on the Gillespie algorithm [25,26]. In simulations, we begin with an initial number of various molecular species. The key reactions in our simulation include synthesis, degradation, complex association/dissociation and transcriptional interactions between the DNA and the transcription factor. The reactions considered here are listed in “Appendix D”. In stochastic simulations, an event or a reaction and the interval between two successive reactions are chosen probabilistically. Based on the reaction that takes place, at each simulation step, the number of molecular species is updated accordingly. We allow the system to evolve for \( 5 \times 10^7 \) steps and record the target protein number after every 500 steps leaving about initial \( 2 \times 10^5 \) steps. The coefficient of variation averaged over 100 samples has been presented in Fig. 5. For this figure \( r_s = 1 \).

Moments necessary for this derivation in terms of lower-order moments using Gaussian approximation [24]. The results for moments up to second order and the approximate forms of the required third-order moments are presented in “Appendix B2”.

Moments required for the evaluation of the coefficient of variation are found numerically using Mathematica. The coefficient of variation as a function of \( r_{m_1} \) for different values of \( r_s \), the sRNA synthesis rate, is shown in Fig. 5. It is clear that the relative fluctuation in the target protein concentration is minimum over a range of \( r_{m_1} \). Interestingly, this minimum region overlaps with the sensitive region shown in Fig. 2. As \( r_s \) increases, the sensitive region extends towards larger \( r_{m_1} \). Figure 5 shows that the minimum fluctuation region also changes accordingly, and there is a systematic increase in the fluctuation beyond this region. In order to see the role of crosstalk in noise processing characteristics, we have also obtained the coefficient of variation for the target protein number for sFFL with \( \gamma_1 = \gamma_2 = 0 \) (no crosstalk) and sCN (see “Appendices B2 and C”). The coefficients of variation plotted with \( r_{m_1} \) indicate the absence of an optimum in noise attenuation (see Figs. 12 and 13 in the “Appendix”).

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Our conclusions on how the target protein concentration as well as its relative fluctuation around the mean value change with \( r_{m_1} \) can be summarized through a single plot as shown in Fig. 6. This plot clearly shows that the range of \( r_{m_1} \) over which the target protein concentration reaches its maximum also corresponds to the range where the relative fluctuation in the target protein level is the least. In order to have further insights on this, we plot absolute fluctuations, \( (\langle p_2^2 \rangle - \langle p_2 \rangle^2)^{1/2} \), with \( r_{m_1} \). Figure 11 of “Appendix B2” shows a rise in the noise level near the sensitive region. The increase in absolute fluctuations is however small compared to the increase in the average target protein concentration leading to an overall minimum in relative fluctuations across the sensitive region.

3 Discussion

Small non-coding RNAs (sRNAs) in bacterial cells are major regulators driving a number of biological processes such as stress response, biofilm formation, and
quorum sensing, under different kinds of environmental signals. It is commonly found that one species of sRNA can regulate translation of several species of mRNAs and such sharing of sRNAs by several co-regulated mRNA targets may have significant effects on gene expression. If the concentration of one species of mRNA, say $m_1$ increases, the sRNAs are expected to bind to $m_1$ predominantly due to its increased concentration. This leads to an increase in sRNA–mRNA1 complex ($c_1$) concentration and consequently a lowering of available free sRNAs and an increase in the concentration of other mRNA targets. The sharing of sRNA thus provides a link between its target mRNAs leading to an sRNA-mediated effective interaction (crosstalk) between the mRNAs. Mostly this link extends over mRNAs from different networks, and the regulation through sRNA sharing may give rise to the possibility of integrating biological processes governed by different networks. In the present work, we are interested in understanding the effect of sRNA-mediated crosstalk between two species of mRNAs in a network motif comprised of a feed-forward loop driven by an sRNA. Thus unlike earlier work, our focus is on the effect of sRNA sharing on the target protein synthesis in a single network motif.

The present study is motivated by a recent finding of an sRNA-driven feed-forward loop in Salmonella enterica subjected to stress due to bile salt. In this network motif, the top-tier regulator is an sRNA (s), RprA, that regulates the target protein ($p_2$), RicI, synthesis along two pathways. While one pathway involves a direct translational activation of RicI protein by RprA, the other pathway involves an indirect activation of RicI expression via translational activation of its transcriptional activator ($p_1$), $\sigma^s$, by RprA. The sRNA, RprA, thus translationally activates its two target mRNAs, $\sigma^s$ mRNA ($m_1$) and RicI mRNA ($m_2$). Such an sRNA-driven feed-forward loop (sFFL) is found to play a significant role in regulating horizontal gene transfer when the bacteria is subjected to stress due to membrane damaging activities of a bactericidal agent, bile salt. Since the horizontal gene transfer is an energy-expensive process, inhibiting such processes during stress conditions might be a preferred strategy for additional protection of the bacteria. RicI protein participates in the stress response activity by interfering with pilus formation which is necessary for bacterial conjugation during horizontal gene transfer.

The $\sigma^s$ protein ($p_1$) which is a transcriptional activator of RicI ($p_2$) is known to be a key stress response regulator that responds to various other environmental stress conditions too. The AND-gate logic of the network motif ensures that the up-regulation of $\sigma^s$ protein ($p_1$) does not necessarily imply an up-regulation of RicI protein ($p_2$) [18]. The present analysis further shows that as a result of the sRNA-mediated crosstalk, the network also leads to an interesting regulation in the target protein synthesis that strongly depends on $m_3$. The effect of crosstalk is appreciated by changing the synthesis rate ($r_{m_1}$) of mRNAs of the intermediate regulator. As $r_{m_1}$ is increased, the concentration of free sRNAs (s) decreases as a consequence of sRNA–mRNA1 complex formation. Due to the reduced availability of free sRNA molecules for binding, the level of free $m_2$ increases. This effect of crosstalk between the two species of mRNAs is more drastic over a narrow range of $r_{m_1}$ which we refer as a sensitive region. In this region, a small increase in the synthesis rate gives rise to a sharp drop in the sRNA concentration along with a steep rise in the concentrations of the two mRNAs. Our major observations are primarily around the sensitive region. In order to see the effect of crosstalk on the target protein synthesis, we have considered two different scenarios here; (i) the target protein is synthesized only from the bound sRNA–mRNA2 complex, (ii) in addition to (i), the target protein is also synthesized through direct translation of its mRNA without sRNA facilitating the ribosome binding. We find that in case of (i), with the increase in $r_{m_1}$, the target protein concentration reaches a maximum and then decreases sharply as a consequence of the sharp drop in free sRNA in the sensitive region. Although target protein mRNAs are synthesized, the translation is less probable due to less availability of free sRNA molecules in the sensitive region. These results can be compared with sFFLs without the stoichiometric decay of the bound complexes ($\gamma_1 = \gamma_2 = 0$) in which case the sRNA mediated crosstalk effect disappears. In this case, instead of a maximum, the target protein level approaches a saturation value as $r_{m_1}$ is increased. In case of scenario (ii), the sharp decrease in the target protein concentration, as seen in (i) beyond the maximum, disappears and due to direct translation of target protein mRNA, the target protein level continues to remain high. In this case, the effect of crosstalk is best appreciated when the results are compared with those of sRNA-driven cascade network (sCN) which is obtained from sFFL by disregarding the possibility of translational activation of the target protein mRNA by sRNA. Since in sCN, sRNAs only activate the transcriptional activator of the target protein, sRNA sharing and hence the sRNA-mediated crosstalk between mRNAs are absent. We find that while in sCN, the target protein concentration increases gradually with $r_{m_1}$, in the case of sFFL (in scenario (ii)), the target protein synthesis shows a threshold response with $r_{m_1}$. Further, as mentioned before, the target protein concentration continues to remain high beyond the threshold. It appears that in sFFL with direct translation of mRNA, the target protein synthesis in the low-concentration regime is dominated by translation from the bound sRNA–mRNA2 complex, while that beyond the threshold is due to direct translation of $m_2$. The threshold property might be beneficial since a significant target protein synthesis happens only beyond a threshold concentration of the intermediate mRNA, thereby prohibiting the cell from a strong stress response under mild stress.

Gene expression is intrinsically noisy with noise originating from the randomness associated with various molecular interactions. This raises a fundamental question as to how cells perform in a robust manner despite significant variations in gene expression levels. Recent
evidences suggest that the architecture of the regulatory network determines the effect of gene expression noise on the target protein level, with some architectures leading to noise amplification, etc. It is believed that the network motifs are evolutionarily selected based on the noise processing characteristics the network architecture may lead to. In view of this knowledge, we attempt to find the interplay of crosstalk and gene expression noise in sFFL and its implications on fluctuations in the target protein level. To this end, we use a generating function-based method to obtain the coefficient of variation (relative fluctuations) in the target protein level. For simplicity, we consider the sFFL where the target protein is synthesized only from the bound complex of sRNA and target protein mRNA. We find that the absolute fluctuation in the target protein level i.e., \( (\langle p_2^2 \rangle - \langle p_2 \rangle^2)^{1/2} \) increases with \( r_{m_1} \) in the sensitive region. This increase, however, is small compared to the increase in the target protein level leading to an overall minimum in relative fluctuations across the sensitive region. We find a remarkable agreement with results from stochastic simulations based on the Gillespie algorithm. These results as well as a comparison with sFFL without crosstalk (\( 1 = 2 = 0 \)) prompt us to conclude that crosstalk can lead to maximum protein synthesis with maximum noise buffering in the sensitive region.

We believe that these results are robust since they appear as direct consequences of the sensitive region present, in general, as a result of the sRNA-mediated crosstalk. All these features resulting primarily from sRNA-mediated crosstalk between mRNAs indicate a complex and precise level of gene regulation through sRNA. Although the present study is motivated by an experimentally observed network, the mathematical framework and the results are general and not restricted to specific parameter values.

In conclusion, the network motif, sFFL, shows different regimes of regulation. The crosstalk leads to a particularly interesting sensitive regime where we find a threshold response or a maximum in the target protein level depending on the explicit mechanism of target protein translation. How one or more of these regimes benefit cellular regulation remains an open question. Future experimental studies along these directions may help address some of the questions.

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Author contribution statement

S.T. and S.M. developed the theoretical formalism, performed the analytical calculations and numerical simulations and jointly wrote the manuscript.

Appendix

Appendix A: Deterministic analysis

1. sRNA-driven cascade network (sCN)

In this section, we consider the sRNA-driven cascade network (sCN) where sRNA translationally activates protein \( p_1 \) which then transcriptionally activates protein \( p_2 \) (see Fig. 7b). Thus, it is a cascade network where no sharing of free sRNA takes place. The variation in concentrations of various components of sCN with time is as follows:

\[
\begin{align*}
\dot{s} &= r_s - \gamma_s s - k_1^s s m_1 + (k_1^c + \kappa_1) c_1, \\
\dot{m}_1 &= r_{m_1} - \gamma_{m_1} m_1 - k_1^s s m_1 + k_1^c c_1, \\
\dot{c}_1 &= k_1^c m_1 s - (k_1^c + \gamma_1 + \kappa_1) c_1, \\
\dot{p}_1 &= r_{p_1} c_1 - \gamma_{p_1} p_1, \\
\dot{m}_2 &= r_{m_2} k_2 p_1 - \gamma_{m_2} m_2 \quad \text{and} \\
\dot{p}_2 &= r_{p_2} m_2 - \gamma_{p_2} p_2.
\end{align*}
\]

The steady-state concentrations of mRNAs \( m_1 \) and \( m_2 \) in terms of functions \( F_1(s) \) and \( F_2(s) \) are

\[
m_1^* = \frac{r_{m_1}}{\gamma_{m_1}}, \quad m_2^* = \frac{r_{m_2}}{\gamma_{m_2}} \quad \text{and} \quad a = \frac{r_{p_1} k_2}{\gamma_{p_1} (k_1^c + \gamma_1 + \kappa_1)}, \\
F_1(s) = \frac{1}{1 + s/s_{01}} \quad \text{and} \quad s_{01} = \frac{\gamma_{m_1}}{k_1^c} \frac{k_1^c + \gamma_1 + \kappa_1}{\gamma_1 + \kappa_1}. \]

In the steady-state, the concentration of sRNA, can be found by solving \( r_s - \gamma_s s - r_{m_1} \gamma s_1 F_1(s) = 0 \), where \( \gamma_1 = \frac{k_1^c}{\gamma_{m_1} (k_1^c + \gamma_1 + \kappa_1)} \). The target protein concentration for sCN is \( p_2^* = \frac{r_{p_2} m_2^*}{\gamma_{p_2}} \) where \( m_2^* \) is as shown in Eq. (A8).

2. Response functions for protein

The response functions for the intermediate and target proteins can be calculated, to understand the sensitivity of proteins, \( (p_1 \text{ and } p_2) \) with respect to the change in the synthesis

\[\text{Post-transcriptional activation}\]

\[\text{Transcriptional activation}\]

\[\text{Protein}\]

\[\text{sRNA}\]

\[\text{Fig. 7 Schematic diagrams for a sFFL and b sCN}\]
and \( r_{m1} \), we find are of concentrations of various regulatory molecules with time

In this section, we consider the sRNA-driven feed-forward loop (sFFL) with the translation of \( p_2 \) from \( m_2 \) without the intervention of sRNA. This direct synthesis of \( p_2 \) from \( m_2 \) is in addition to translation facilitated by sRNA through formation of sRNA–mRNA2 complex (\( c_2 \)). The variation of concentrations of various regulatory molecules with time are

\[
\begin{align*}
\dot{s} &= r - \gamma_s s - k^+_1 s m_1 - k^+_2 s m_2 + (k^-_1 + \kappa_1) c_1 + (k^-_2 + \kappa_2) c_2, \\
\dot{m}_1 &= r_{m1} - \gamma_{m1} m_1 - k^+_1 s m_1 + k^-_1 c_1, \\
\dot{c}_1 &= k^+_1 s m_1 - (k^-_1 + \gamma_1 + \kappa_1) c_1, \\
\dot{p}_1 &= r_{p1} c_1 - \gamma_{p1} p_1,
\end{align*}
\]

\( \dot{m}_2 = \frac{r_{m2} \kappa_2 p_1}{1 + \kappa_2 p_1} - \gamma_{m2} m_2 - k^+_2 s m_2 + k^-_2 c_2, \]

\( \dot{c}_2 = k^+_2 s m_2 - (k^-_2 + \gamma_2 + \kappa_2) c_2 \) and

\( \dot{p}_2 = r_{p2} c_2 + r_{p20} m_2 - \gamma_{p2} p_2, \)

where \( r_{p20} \) represents the rate of synthesis of the target protein, \( p_2 \) from \( m_2 \) without the involvement of sRNA.

Figure 9a shows that there is similar crosstalk behaviour as shown in Fig. 2. However, unlike \( r_{p20} = 0 \) case, here, due to direct synthesis of the target protein, the \( p_2 \) concentration remains high even beyond the peak (see Fig. 9b). This region appears to be \( r_{p20} \) dominated. The overlap of \( p_2 \) plots for different \( r_{p20} \) values before the sensitive region indicates that the target protein synthesis happens here predominantly due to bound complexes.
4. Effect of different factors on target protein synthesis

In this section, we study the change in the target protein (p2) concentration with r_{m1} for different synthesis rates (r_{m2}) of mRNA2 with (i) nonzero stoichiometric as well as catalytic decay rates (Fig. 10a) (ii) only catalytic decay i.e., γ_1 = γ_2 = 0 and κ_1, κ_2 ≠ 0 (Fig. 10b) and (iii) with only stoichiometric decay and no catalytic decay i.e., κ_1 = κ_2 = 0 and γ_1, γ_2 ≠ 0 (Fig. 10c). For all the figures, r_{p20} = 0. As expected, all the figures show a strong dependence on r_{m2}. With the increase in r_{m2}, the target protein concentration increases naturally. Further, the increase in m_2 concentration leads to an increased competition for the available free sRNAs which finally leads to reduced plateau region. Figure 10d displays a comparison of the three cases, namely (i) no crosstalk i.e., γ_1 = γ_2 = 0 and κ_1, κ_2 ≠ 0, (dotted line) (ii) with crosstalk i.e., γ_1, γ_2 ≠ 0 and κ_1, κ_2 ≠ 0 (solid line) and (iii) with crosstalk but without sRNA recycling i.e., κ_1 = κ_2 = 0 and γ_1, γ_2 ≠ 0 (dashed line).

Appendix B: Noise analysis for sFFL

1. Equations and notations

The reactions incorporated into the master equation, Eq. (21), are based on the following effective equations.

\[
\dot{s} = r_s - γ_s s - g_1 s m_1 - g_2 s m_2, \quad (B1)
\]

\[
\dot{m}_1 = r_{m1} - γ_{m1} m_1 - d_1 s m_1 - g_1 s m_1, \quad (B2)
\]

\[
P_1 = \frac{r'_{p1}}{k_1} s m_1 - γ_{p1} P_1, \quad (B3)
\]

\[
\dot{m}_2 = \frac{r_{m2} k_2 P_1}{1 + k_c P_1} - γ_{m2} m_2 - d_2 s m_2 - g_2 s m_2 \quad \text{and} \quad (B4)
\]

\[
P_2 = \frac{r'_{p2}}{k_2} s m_2 - γ_{p2} P_2. \quad (B5)
\]

As mentioned in the main text, a quasi-steady-state approximation has been used in obtaining these equations. In this approximation, we assume that the process of complex formation attains steady state faster compared to other processes. Using \( c_1 = \frac{k_1^+ s m_1}{k^{-1}_1 + γ_1 + κ_1} \) and \( c_2 = \frac{k_2^+ s m_1}{k^{-1}_2 + γ_2 + κ_2} \) in Eqs. (1)–(7), we find the effective equations mentioned above [Eqs. (B1)–(B5)]. Here, \( g_1 = \frac{k_1^+ γ_1}{k_1 + γ_1 + κ_1} \) and \( g_2 = \frac{k_2^+ γ_2}{k_2 + γ_2 + κ_2} \) correspond to combined degradation of sRNA and mRNA and \( d_1 = \frac{k_1^+ κ_1}{k_1^{-1} + γ_1 + κ_1} \) and \( d_2 = \frac{k_2^+ κ_2}{k_2^{-1} + γ_2 + κ_2} \) indicate degradation of mRNA alone, while sRNAs are recycled back. With the quasi-steady-state approximation, the effective protein synthesis rates are \( r'_{p1} = \frac{r_{p1} k_1^+}{k_1^{-1} + γ_1 + κ_1} \) and \( r'_{p2} = \frac{r_{p2} k_2^+}{k_2^{-1} + γ_2 + κ_2} \).

2. Moments

In this section, we list the results for first- and second-order moments obtained from the generating function approach. The results show that moments of a given order involve higher-order moments. In order to simplify our calculations, we consider moments up to second order and express the

![Fig. 10](image-url) Variation in the target protein concentration (p2) of sFFL with r_{m2}, a for different r_{m2} values with γ_1 = γ_2 = κ_1 = κ_2 = 0.01, b for different r_{m2} values with γ_1 = γ_2 = 0 and κ_1 = κ_2 = 0.01, c for different r_{m2} values with γ_1 = γ_2 = 0.01 and κ_1 = κ_2 = 0 and d for r_{m2} = 1 under different conditions like with crosstalk i.e., γ_1 = γ_2 = 0.01 and κ_1 = κ_2 = 0.01, without crosstalk i.e., γ_1 = γ_2 = 0 and κ_1 = κ_2 = 0.01 and without recycling of sRNA i.e., κ_1 = κ_2 = 0 and γ_1 = γ_2 = 0.01. The other parameter values are the same as those in Fig. 2.
third-order moments in terms of lower-order moments using Gaussian approximation.

\[ G_1 = r_s - g_1 G_{12} - g_2 G_{14} \]  
\[ G_2 = \frac{r_{m_1} - (g_1 + d_1) G_{12}}{\gamma_m} \]  
\[ G_3 = \frac{r'_{p_1} G_{12}}{\gamma_{p_1}} \]  
\[ G_4 = \frac{r_{m_1} + r'_{m_1} G_3 - (g_2 + d_2) G_{14}}{\gamma_m} \]  
\[ G_5 = \frac{r'_{p_1} G_{14}}{\gamma_{p_1}} \]  
\[ G_{11} = \frac{r_s G_3 - g_1 (G_{12} + G_{122}) - g_2 G_{124} - d_1 G_{112}}{\gamma_s + \gamma_m + g_1 + d_1} \]  
\[ G_{12} = \frac{r_s G_4 + r'_{p_1} (G_{14} + G_{114}) - g_1 G_{125} - g_2 G_{145}}{\gamma_s + \gamma_{p_1}} \]  
\[ G_{13} = \frac{r_s G_4 + r_{m_1} G_3 + r'_{m_1} G_3 - (g_2 + d_2) G_{14} - g_2 G_{145}}{\gamma_s + \gamma_m + g_1 + d_1} \]  
\[ G_{14} = \frac{r_s G_4 + r_{m_1} G_3 + r'_{m_1} G_3 - (g_2 + d_2) G_{14} - g_2 G_{145}}{\gamma_s + \gamma_m + g_1 + d_1} \]  
\[ G_{23} = \frac{r_{m_1} G_4 + r'_{m_1} G_3 + r'_{m_1} G_3 - (g_2 + d_2) G_{14} - g_2 G_{145}}{\gamma_s + \gamma_m + g_1 + d_1} \]  
\[ G_{24} = \frac{r_{m_1} G_4 + r'_{m_1} G_3 + r'_{m_1} G_3 - (g_2 + d_2) G_{14} - g_2 G_{145}}{\gamma_s + \gamma_m + g_1 + d_1} \]  
\[ G_{25} = \frac{r_{m_1} G_5 + r'_{m_1} G_{122} - (g_1 + d_1) G_{125}}{\gamma_m + \gamma_{p_1}} \]  
\[ G_{34} = \frac{r'_{p_1} G_{122} + r_{m_1} G_3 + r'_{m_1} G_3 - (g_2 + d_2) G_{14}}{\gamma_{p_1} + \gamma_m} \]  
\[ G_{35} = \frac{r'_{p_1} G_{125} + r_{p_1} G_{134}}{\gamma_{p_1}} \]  
\[ G_{45} = \frac{r_{m_1} G_5 + r'_{m_1} G_{35} + r'_{m_1} (G_{14} + G_{144}) - (g_2 + d_2) G_{145}}{\gamma_s + \gamma_{p_1}} \]  

Using Gaussian approximation, we express the third-order moments in terms of the lower-order moments as shown below.

\[ G_{112} = G_{11} G_2 + G_1 G_2 + 2G_1 G_{12} - 2G_1^2 G_2 - G_{12} \]  
\[ G_{113} = G_{11} G_3 + G_1 G_3 + 2G_1 G_{13} - 2G_1^2 G_3 - G_{13} \]  
\[ G_{114} = G_{11} G_4 + G_1 G_4 + 2G_1 G_{14} - 2G_1^2 G_4 - G_{14} \]  
\[ G_{122} = G_{22} G_1 + G_1 G_2 + 2G_2 G_{12} - 2G_2^2 G_1 - G_{12} \]  

In order to see the effect of crosstalk, we have plotted the coefficient of variation for the target protein with \( r_{m_1} \) as a function of the parameter values used here. As Fig. 11 shows, the absolute fluctuations in the target protein, \( \langle (y_2^2) - \langle y_2 \rangle^2 \rangle^{1/2} \), increase in the sensitive regime. In the absence of crosstalk (i.e., for \( \gamma_1 = \gamma_2 = 0 \)), the coefficient of variation is significantly different from that of the Gaussian approximation (see Fig. 12).
Appendix C: Noise analysis for sCN

In the case of sCN, we follow the same master equation approach as done for sFFL. The master equation for the probability of a given state is based on the following effective differential equations

\[ \dot{s} = s - \gamma_s s - g_1 s m_1, \]  
\[ \dot{m}_1 = r_{m_1} - \gamma_{m_1} m_1 - d_1 s m_1 - g_1 s m_1, \]  
\[ \dot{p}_1 = r_{p_1} s m_1 - \gamma_{p_1} p_1, \]  
\[ \dot{m}_2 = r_{m_2} k_0 p_1 - \gamma_{m_2} m_2 \text{ and } \]  
\[ \dot{p}_2 = r_{p_2} m_2 - \gamma_{p_2} p_2. \]  

In Fig. 13, we plot the coefficient of variation \( CV_{p_2} = (G_{55} + G_5 - G_5^2)^{1/2} / G_5 \) with \( r_{m_1} \). No minimum in the coefficient of variation is found in this case. Here, \( r_s = 1 \) and all other parameter values are the same as those in Fig. 5. In the following, we present first and second moments necessary for obtaining the coefficient of variation for the sRNA-driven cascade network. As before, we use Gaussian approximation to express the third moments in terms of various first and second moments.

\[ G_1 = \frac{r_s - g_1 G_{12}}{\gamma_s} \]  
\[ G_2 = \frac{r_{m_1} - (g_1 + d_1) G_{12}}{\gamma_{m_1}} \]  
\[ G_3 = \frac{r_{p_1} G_{12}}{\gamma_{p_1}} \]  
\[ G_4 = \frac{r_{m_2}^0 + r_{m_2}^1 G_3}{\gamma_{m_2}} \]  
\[ G_5 = \frac{r_{p_{20}} G_4}{\gamma_{p_2}} \]  
\[ G_{11} = \frac{r_s G_1 - g_1 G_{112}}{\gamma_s} \]  
\[ G_{22} = \frac{r_{m_1} G_2 - (g_1 + d_1) G_{122}}{\gamma_{m_1}} \]  
\[ G_{33} = \frac{r_{p_1} G_{123}}{\gamma_{p_1}} \]  
\[ G_{44} = \frac{r_{m_2} G_4 + r_{m_2}^1 G_{44}}{\gamma_{m_2}} \]  
\[ G_{55} = \frac{r_{p_{20}} G_{45}}{\gamma_{p_2}} \]  
\[ G_{12} = \frac{r_s G_2 + r_{m_1} G_1 - g_1(G_{112} + G_{122}) - d_1 G_{112}}{\gamma_s + \gamma_{m_1} + g_1 + d_1} \]  
\[ G_{13} = \frac{r_s G_3 + r_{p_1} (G_{12} + G_{112}) - g_1 G_{123}}{\gamma_s + \gamma_{p_1}} \]  
\[ G_{14} = \frac{r_s G_4 + r_{m_2} G_1 + r_{m_2}^1 G_{13} - g_1 G_{124}}{\gamma_s + \gamma_{m_2}} \]  
\[ G_{15} = \frac{r_s G_5 + r_{p_{20}} G_{14} - g_1 G_{125}}{\gamma_s + \gamma_{p_2}} \]  
\[ G_{23} = \frac{r_{m_1} G_3 + r_{p_1}' (G_{12} + G_{122}) - (g_1 + d_1) G_{123}}{\gamma_{m_1} + \gamma_{p_1}} \]  
\[ G_{24} = \frac{r_{m_1} G_4 + r_{m_2} G_2 + r_{m_2}^1 G_{23} - (g_1 + d_1) G_{124}}{\gamma_{m_2} + \gamma_{m_2}} \]  
\[ G_{25} = \frac{r_{m_1} G_5 + r_{p_{20}} G_{24} - (g_1 + d_1) G_{125}}{\gamma_{m_1} + \gamma_{p_2}} \]  
\[ G_{34} = \frac{r_{p_1}' G_{123} + r_{m_2}^0 G_{3} + r_{m_2}^1 (G_{3} + G_{33})}{\gamma_{p_1} + \gamma_{m_2}} \]  
\[ G_{35} = \frac{r_{p_1}' G_{125} + r_{p_{20}} G_{34}}{\gamma_{p_1} + \gamma_{p_2}} \]  
\[ G_{45} = \frac{r_{m_2}^0 G_5 + r_{m_2}^1 G_{35} + r_{p_{20}} (G_4 + G_{44})}{\gamma_{m_2} + \gamma_{p_2}} \]
Appendix D: Stochastic simulations

The reactions considered for the stochastic simulations and the corresponding rates are listed below.

\[
\begin{align*}
\phi & \xrightarrow{r_s} s \quad \text{(synthesis and degradation of sRNA (s); } r_s = \text{variable, } \gamma_s = 0.001 \text{ (s}^{-1})], \\
\phi & \xrightarrow{r_{m_1}} m_1 \quad \text{(synthesis and degradation of mRNA1 (m_1); } r_{m_1} = \text{variable, } \gamma_{m_1} = 0.001 \text{ (s}^{-1})], \\
m_1 + s & \xrightarrow{k_1^+ \over k_1^-} c_1 \quad \text{association and dissociation of sRNA-mRNA1 complex (c_1); } k_1^+ = 0.1 \text{ (molecules}\cdot\text{s}^{-1}), k_1^- = 0.05 \text{ (s}^{-1}), \\
c_1 & \xrightarrow{\gamma_1} \phi \quad \text{(stoichiometric degradation of sRNA-mRNA1 complex (c_1); } \gamma_1 = 0.005 \text{ (s}^{-1})], \\
c_1 & \xrightarrow{\kappa_1} s \quad \text{(catalytic degradation of sRNA-mRNA1 complex (c_1); } \kappa_1 = 0.005 \text{ (s}^{-1})], \\
c_1 & \xrightarrow{r_{p_1}} p_1 + c_1 \quad \text{(translation and synthesis of protein-1 (p_1); } r_{p_1} = 0.01 \text{ (molecules. s}^{-1})], \\
p_1 & \xrightarrow{\gamma_{p_1}} \phi \quad \text{(degradation of protein-1 (p_1); } \gamma_{p_1} = 0.001 \text{ (s}^{-1})], \\
p_1 + G_{p_2} & \xrightarrow{k_2^+ \over k_2^-} G_{p_2}^* \quad \text{transcriptional activation of gene synthesizing protein-2 (p_2); } k_2^+ = 0.2 \text{ (molecules}\cdot\text{s}^{-1}, k_2^- = 2 \text{ (s}^{-1}), \\
G_{p_2}^* & \xrightarrow{r_{m_2}} m_2 + G_{p_2}^* \quad \text{(synthesis of mRNA2 (m_2); } r_{m_2} = 0.01 \text{ (molecules. s}^{-1})], \\
m_2 + s & \xrightarrow{k_2^+ \over k_2^-} c_2 \quad \text{association and dissociation of sRNA-mRNA2 complex (c_2); } k_2^+ = 0.1 \text{ (molecules}\cdot\text{s}^{-1}, k_2^- = 0.05 \text{ (s}^{-1}), \\
c_2 & \xrightarrow{\gamma_2} \phi \quad \text{(stoichiometric degradation of sRNA-mRNA2 complex (c_2); } \gamma_2 = 0.005 \text{ (s}^{-1})], \\
c_2 & \xrightarrow{\kappa_2} s \quad \text{(catalytic degradation of sRNA-mRNA2 complex (c_2); } \kappa_2 = 0.005 \text{ (s}^{-1})], \\
m_2 & \xrightarrow{\gamma_{m_2}} \phi \quad \text{(degradation of mRNA2 (m_2); } \gamma_{m_2} = 0.001 \text{ (s}^{-1})], \\
c_2 & \xrightarrow{r_{p_2}} p_2 + c_2 \quad \text{(translation and synthesis of protein-2 (p_2); } r_{p_2} = 0.01 \text{ (molecules. s}^{-1}) \text{ and } \\
p_2 & \xrightarrow{\gamma_{p_2}} \phi \quad \text{(degradation of protein-2 (p_2); } \gamma_{p_2} = 0.001 \text{ (s}^{-1})].
\end{align*}
\]
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