CHAPTER 3

INFORMATION TRANSMISSION IN NETWORKS WITH FEED-FORWARD LOOPS OR DIAMOND MOTIFS

Using a Gaussian model, we study the transmission of time-varying biochemical signals through feed-forward motifs and diamond motifs. To this end, we compute the frequency dependence of the gain, the noise, as well as their ratio, the gain-to-noise ratio, which measures how reliably a network transmits signals at different frequencies. We find that both coherent and incoherent feed-forward motifs can either act as low-pass or high-pass filters for information: the frequency dependence of the gain-to-noise ratio increases or decreases with increasing frequency, respectively. Our analysis of diamond motifs reveals that cooperative activation of the output component can increase the gain-to-noise ratio. This means that from the perspective of information transmission, it can be beneficial to split the input signal in two and recombine the two propagated signals at the output. Cooperative activation can be implemented via the formation of homo- or heteromultimers that then bind and activate the output component or via the binding of individual molecules of the intermediate species to the output component.

Based on manuscript W.H. de Ronde, F. Tostevin and P.R. ten Wolde. Feed-forward loops and diamond motifs allow for tunable information transmission Submitted.
3.1 Introduction

Cells live in a highly dynamic environment. While in some cases cells may wish to ignore rapid fluctuations and only respond to persistent changes, in other cases they may have to do the opposite. For example, in the case of chemotaxis or osmo-adaptation, cells respond to changes in the stimulus but are insensitive to the absolute level of the stimulus. In contrast, in response to a changing sugar concentration, cells respond to the absolute steady-state sugar level, but may wish to integrate out rapid fluctuations of the sugar level. In general, to understand how cells cope with a changing environment, we have to understand how cells transduce time-varying signals. Moreover, given the observation that the biochemical networks which process the signals are stochastic in nature, we have to understand how reliably biochemical networks can process time-varying signals in the presence of noise.

Cells use recurring network motifs to specifically respond to temporal characteristics of the input signal. Negative feedback or incoherent feed-forward loops may be used to only respond to rapid variations and not to slow changes in the environment [101], while coherent feed-forward loops can be used to filter out transient fluctuations in the input and only respond to persistent changes in the environment [106]. To understand how specifically and reliably these motifs can respond to inputs with distinct temporal dynamics, we have to understand how they amplify input signals as a function of their frequency [65], which is characterized by the frequency-dependent gain. Moreover, we have to understand how they propagate biochemical noise as a function of frequency [65]. Indeed, information theory [45] tells us that the fidelity by which a signal of a given frequency is transmitted, is determined by the gain-to-noise ratio at that frequency [65]. We have recently shown for motifs with different types of feedback regulation that different network architectures affect the frequency dependence of the gain and the noise differently [107], which means that both of these quantities have to be studied together in order to understand how reliably a network transmits time-varying signals.

In this chapter, we use a Gaussian model to study the frequency dependence of the gain, noise and gain-to-noise ratio [65, 107] of feed-forward loops and diamond motifs. Both are common motifs in biochemical networks [11, 98]. Feed-forward motifs have been shown to regulate many different cellular processes, and, indeed, they exhibit very rich dynamics. Feed-forward motifs can act as sign-sensitive circuits [17], perform adaptation [101], provide fold-change detection [108] or attenuate extrinsic noise [109].

While the mean-field response [11, 17, 98, 101, 108, 109] and the noise characteristics [37, 41] of feed-forward loops and diamond motifs have been well characterized, how reliably they propagate time-varying signals has not been studied. This is of specific interest since the data-processing inequality dictates that information transmission decreases with the length of the cascade. If information transmission is the only constraint, the shortest cascade is the most reliable solution. However, as discussed above, cells may wish to respond to specific frequencies in the input signal and it may have to do so reliably. This is precisely what feed-forward loops and diamond motifs can achieve, in contrast to simple cascades.

We find that coherent feed-forward motifs and diamond motifs typically act as low pass filters for information: they transmit input signals of low frequency more reliably than input signals of high frequency. In contrast, incoherent feed-forward motifs tend to act as
3.2 Methods

In this section, we briefly discuss the mathematical background of our analysis. A more in depth analysis is presented in [65, 67, 107]. The biochemical network consists of the components $S, V_i$ (intermediate(s)) and $X$. Here $S$ is the input and $X$ is the output. We model the time evolution of these components using a set of coupled Langevin equations [69], which can be non-linear, e.g:

\[
\frac{ds}{dt} = f_s(s) - \mu_s s + \Gamma(t),
\]

\[
\frac{dv_i}{dt} = f_{v_i}(s, v) - \mu_{v_i} v_i + \eta_{v_i}(t),
\]

\[
\frac{dx}{dt} = f_x(s, v, x) - \mu_x x + \eta_x(t).
\]

For simplicity, we assume linear degradation of each component. The various noise sources ($\eta_i, \eta_x$) in Eqs. 3.2.3.3 are assumed to be independent and Gaussian distributed [31, 77, 96]. We take the noise strength ($\langle |\eta_i|^2 \rangle$, $\langle |\eta_x|^2 \rangle$) as the sum of the average number of production and degradation events per unit amount of time for component $v_i, x$ [68, 70, 97]. We assume the noise source $\Gamma(t)$ to be a Gaussian white noise. It generates an ensemble of input trajectories $s(t)$ with Gaussian statistics. The “forces” $f_{s,v_i,x}(s,v,x)$ model all the reactions involving the production events of $s, v_i$ and $x$.

We assume that the network has a steady state and linearize about this steady state, so that we get a dynamical equation for the fluctuations of each component, $\tilde{v}_i(t) = v_i(t) - \langle v_i \rangle$, and similarly for the input $s$ and output $x$. In the linearized form, the relation between the components $i, j$ is described by the coupling coefficient $J$, which are the Jacobian
elements, e.g.

\[ J_{v_i v_j} = \frac{\partial^2 d_v}{\partial v_i \partial v_j} = -\mu v_i, \quad J_{x v_i} = \frac{\partial^2 d_x}{\partial v_i}. \] (3.4)

We take as the input signal the variations of \( \tilde{s} (t) \) around its mean \( \langle s \rangle \), \( \tilde{s} (t) \), and as the output the variations \( \tilde{x} (t) \) of \( x (t) \) around its mean \( \langle x \rangle \). The mutual information rate between the in- and output trajectories, \( s(t) \) and \( x(t) \) respectively, is defined as [65]

\[ R [\tilde{s} (t), \tilde{x} (t)] = \frac{1}{2\pi} \int_0^\infty d\omega \ln \left[ 1 + \frac{g^2 (\omega)}{N (\omega)} P_{ss} (\omega) \right], \] (3.5)

where \( P_{ss} \) is the power spectrum of the signal,

\[ P_{ss} (\omega) = \langle \tilde{s} (\omega) \tilde{s} (-\omega) \rangle. \] (3.6)

The gain \( g^2 (\omega) \) and noise \( N (\omega) \) are defined through the power spectra

\[ g^2 (\omega) \equiv \frac{|P_{xx} (\omega)|^2}{P_{ss}^2 (\omega)}, \] (3.7)

\[ N (\omega) \equiv P_{xx} (\omega) - g^2 (\omega) P_{ss} (\omega). \] (3.8)

These definitions are prescribed by using Eq. 3.5. A large gain-to-noise ratio \( g^2 (\omega) / N (\omega) \) (GNR) leads to a high mutual information rate (Eq. 3.5) and this implies reliable information transmission.

We have made a number of assumptions to obtain Eq. 3.5. First, we assume that the linearized system is an accurate representation of the non-linear system. Second, we assume that the variations \( \tilde{s} (t) \) and \( \tilde{x} (t) \) can be described by a Gaussian joint-probability distribution. Third, and last, we assume the signal \( \tilde{s} (t) \) to be modular from the underlying network. Modularity of the signal with respect to the network indicates that no correlations exists between the variations in the input signal and the intrinsic noise of the reactions that constitute the processing network; it also implies that there is no feedback from the network onto the input signal. If signal modularity holds, then Eq. 3.8 is equal to the spectral addition rule [96]. In this case, the gain-to-noise ratio \( g^2 (\omega) / N (\omega) \), does not depend on the input signal, but only on the information transmission characteristics of the processing network; it describes the ability of the network to reliably propagate input signals as a function of their frequency. As an additional simplification we assume that no (anti)-correlations between the different noise sources are present [107]. While these may quantitatively change the results presented below, they do not qualitatively change them. In the next section we will describe the effect of the feed-forward motif on the information transmission through a biochemical network. We will characterize the gain, noise and gain-to-noise ratio, since these are intrinsic, signal-independent, properties of the network, when the spectral-addition rule holds [96]. We compare different motifs with simple linear one-step (\( S \to X \)) and linear two-step cascades (\( S \to V \to X \)). We will compare the different
networks, unless specified otherwise, under the constraint that the average production rates of the respective components are the same in the networks under comparison.

Lastly, we will comment on the differences between the gain-to-noise ratio, which describes information transmission and \( P_{xx} (\omega) \), which describes power transmission.

3.3 Results

3.3.1 Simple Cascades

Simple cascades form the building blocks of the feed-forward motif. The feed-forward motif consists of two cascades, one in which the input S directly regulates the output X via a one-step cascade and one where S indirectly regulates the output X via a two-step cascade with an intermediate component V. A diamond motif consists of two two-step cascades which start and end at the same component, S and X, respectively. Since we will compare the behavior of these networks with simple cascades consisting of one or two steps, it will be useful to briefly recall their main transmission characteristics. A more detailed discussion can be found in [107] and Chapter 2.

For a one-step cascade the gain is given by \( k_x^2 / (\omega^2 + \mu_x^2) \) where \( k_x = J_{xs} \) is the coupling between \( s \) and \( x \) and \( \mu_x \) is the lifetime of \( X \), while the noise is given by \( \langle |\eta_x|^2 \rangle / (\omega^2 + \mu_x^2) \). Consequently, the GNR of a one-step cascade is constant for all frequencies (Eq. A3.5). For the two-step cascade the gain is \( k_x k_y^2 / (\omega^2 + m_y^2)(\omega^2 + m_x^2) \), with two corner frequencies, \( \mu_x, \mu_y \), the noise is \( k_x^2 \langle |\eta_x|^2 \rangle / (\omega^2 + m_y^2)(\omega^2 + m_x^2) \) and as a result the GNR decays with \( \omega^{-2} \) for \( \omega > (\mu_x + k_x \langle |\eta_x|^2 \rangle / \langle |\eta_x|^2 \rangle)^{1/2} \). For more details see App. 3.A.1.

3.3.2 The feed-forward motif

Two different types of feed-forward (ff) motifs exist. If the total regulatory effect of \( S \) on \( X \) and \( S \) via \( V \) on \( X \) are of the same nature, both either active or inactive, the motif is referred to as coherent feed-forward (cff). If the regulatory effects are opposing, the motif is referred to as incoherent feed-forward (iff). For the coherent motif we can further differentiate with respect to the integration strategy at the reporter \( X \). If both \( S \) and \( V \) are required to produce \( X \), the node \( X \) acts as an AND-gate and we refer to the motif as AND coherent feed-forward (acf). If either \( S \) or \( V \) is sufficient to produce \( X \), we refer to the motif as OR coherent feed-forward (ocf). The AND type is observed in the ara system of E. coli [17], while the OR type is present in the biosynthesis of the flagellar motor [110]. The motifs are shown in Figs. 3.1a (ocf), 3.2a (acf) and 3.3a (iff). In this chapter we study an iff motif for which the negative regulation is always at the response \( X \). As a result, a distinction between AND and OR regulation in the iff is not made. We will discuss this assumption in more detail in the section discussing the iff. We now start by studying some general characteristics of the feed-forward motif.
General characteristics

We first study the gain. The gain for the feed-forward is

$$g^2(\omega) = \left( \frac{g^2_{x \rightarrow x}}{\omega^2 + J_{xx}^2} \right) + \left( \frac{g^2_{x \rightarrow v \rightarrow x}}{(\mu_v J_{xx})^2} \right) + \left( \frac{\text{coherence}}{\omega^2 + J_{xx}^2 (\omega^2 + J_{vv}^2)} \right). \quad (3.9)$$

The first term is the gain due to the direct regulation of X by S($g^2_{x \rightarrow x}$), the second term is due to the pathway S → V → X ($g^2_{x \rightarrow v \rightarrow x}$) and the third term is a term due to the interaction between the two pathways.

The first term $g^2_{x \rightarrow x}$ is the gain of a one-step cascade in which the input $s$ regulates the output $x$. It depends on the coupling constant $J_{xx}$ and the lifetime of the protein X, $\mu_x^{-1} = -J_{xx}^{-1}$. If the lifetime $\mu_x^{-1}$ of X is longer than the timescale $\omega^{-1}$ on which the input signal varies, $\omega \gg \mu_x$, then variations in the input $s(t)$ are filtered out by the slow response of X.

The second term $g^2_{x \rightarrow v \rightarrow x}$ is the gain of a two-step cascade in which the input $s$ regulates the output $x$ via an intermediate $v$. It is seen that the gain of the two-step cascade is the product of the gain in each cascade step. This gives rise to two corner frequencies in $g^2_{x \rightarrow v \rightarrow x}$, one at $J_{vv} = -\mu_v$ and another at $J_{xx} = -\mu_x$, where $\mu_v$ and $\mu_x$ are the degradation rates of proteins V and X, respectively. The gain is large for $\omega \ll \mu_v, \mu_x$, since in this frequency regime both V and X can respond rapidly on the time scale of the signal variations, $\omega^{-1}$, while for frequencies $\omega \gg \mu_v, \mu_x$, the gain decreases strongly, scaling as $\omega^{-4}$, because in this regime the input variations are filtered by the finite lifetime of both V and X. Note that a lower degradation rate of the proteins increases the gain at low frequencies, but also reduces the corner frequencies beyond which the gain rapidly drops. This is a generic trade-off between the bandwidth of information transmission (the frequency range, bounded by the corner frequency, with large GNR) and the magnitude of the gain in the band.

The third term describes the coherence of the interaction between the signal transmitted via the direct pathway S → X and the signal transmitted via the indirect pathway S → V → X. Both signals originate from the source signal $s(t)$, which means that their variations are correlated. If $\text{sgn}(J_{xx}) = \text{sgn}(J_{vu} J_{vv})$ the third term is positive (since by construction $J_{vv} = -\mu_v$) and we have a coherent interaction. Such a coherent interaction is present in coherent feed-forward networks and leads to an increase in the gain. If $\text{sgn}(J_{xx}) \neq \text{sgn}(J_{vu} J_{vv})$, as in the incoherent feed-forward motifs, the coherence term is negative and thus the gain $g^2(\omega)$ is decreased.

It is instructive to compare the phase of the direct pathway $\phi_{s \rightarrow x}$ and the indirect pathway ($\phi_{s \rightarrow v \rightarrow x}$) at $x$

$$\phi_{s \rightarrow x} = \phi_s, \quad (3.10)$$

$$\phi_{s \rightarrow v \rightarrow x}(\omega) = \phi_s - \arctan \left( \frac{\omega}{\mu_v} \right). \quad (3.11)$$
3.3 Results

For $\omega = 0$, both signals are in phase. As the frequency increases, the phase of the signal that is transmitted via the indirect pathway decreases with respect to that which is transmitted via the direct pathway. At the corner frequency $-\mu_v = J_{vv}$, the phase difference is $\phi_{s \rightarrow v \rightarrow x} = (J_{vv}) - \phi_s = -\pi/4$ and for $\omega \rightarrow \infty$, the signals are even more out of phase, $\phi_{s \rightarrow v \rightarrow x} - \phi_s = -\pi/2$. Combining both the phase and amplitude information, we see that the coherence between the two signals decreases with increasing $\omega$ for two related reasons. First, there is a decrease in the coherence term for large $\omega$ due to the time-averaging over the fast signal fluctuations resulting from the limited response time of $X$ and $V$. Second, the coherence decreases because the phase difference increases (see App. 3.A.8).

Before we consider the noise, it is useful to briefly consider the three terms of the gain together. While the gain of the direct pathway scales for $\omega \gg \mu_v$ as $\omega^{-2}$, the gain of the indirect pathway and the coherence term scale for $\omega \gg \mu_v, \mu_x$ as $\omega^{-4}$. This means that for frequencies $\omega \gg \mu_v$ the gain of the direct pathway dominates.

The noise in the linearized feed-forward motif is

$$N(\omega) = \frac{N_{v \rightarrow x}(\omega)}{J_{xv}^2 \left\langle |\eta_v|^2 \right\rangle + \left\langle |\eta_x|^2 \right\rangle + \left\langle |\eta_v|^2 \right\rangle} = g_{v \rightarrow x}^2(\omega)N_v(\omega) + \left\langle |\eta_x|^2 \right\rangle \left(\frac{\omega^2 + J_{xv}^2}{\omega^2 + J_{xx}^2}\right),$$

(3.12)

It is seen that the total noise in $x$ is independent of the regulatory effect of either pathway, since all terms are positive. The expression also reveals that the noise is the sum of two noise sources. One is the intrinsic noise arising from the stochastic production and decay of $X$, given by $N_x(\omega) = \left\langle |\eta_x|^2 \right\rangle / (\omega^2 + J_{xx}^2)$. The other is the extrinsic noise coming from the stochastic production and decay of $V$, which is given by the intrinsic noise of $v$, $N_v(\omega) = \left\langle |\eta_v|^2 \right\rangle / (\omega^2 + J_{vv}^2)$, multiplied by a frequency-dependent gain $g_{v \rightarrow x}^2 = J_{xv}^2 / (\omega^2 + J_{xx}^2)$ which reflects how the noise from $v$ is amplified by $J_{xv}$ and integrated by $x$ as a result of its finite lifetime. While the intrinsic noise in $x$, $N_x(\omega)$, scales as $\omega^{-2}$ for $\omega \gg \mu_x = -J_{xx}$, the extrinsic noise $g_{v \rightarrow x}^2(\omega)N_v(\omega)$ scales as $\omega^{-4}$ for $\omega \gg \mu_v, \mu_x$. Indeed, for $\omega \gg \mu_v$, the noise that originates from $v$ in the indirect pathway becomes negligible.

Finally, we obtain the GNR for this three component motif

$$\frac{g^2(\omega)}{N(\omega)} = \frac{\left(\omega^2 + J_{vv}^2\right) J_{xv}^2 + (J_{xv}J_{xx})^2 - 2J_{xv}J_{xv}J_{xx}J_{vv}}{J_{xv}^2 \left\langle |\eta_v|^2 \right\rangle + \left\langle |\eta_x|^2 \right\rangle + \left\langle |\eta_v|^2 \right\rangle}.$$  (3.14)

While the noise is independent of the regulatory effect in the pathways, the gain, and hence the gain-to-noise ratio, depends on the total regulatory effect of each pathway (either $S \rightarrow X$ or $S \rightarrow V \rightarrow X$). This indicates that it is not important which of the reactions in a specific pathway acts negatively, only the overall effect of the pathway is important for information transmission.

We are now in a position to study in more detail the feed-forward motif. As discussed
above, for $\omega \gg \mu_v = -J_{vv}$, the gain of the direct pathway dominates the total gain, because the finite lifetime of $V$ averages out the variations in the signal that are transmitted via the indirect pathway. Also the noise that originates at $v$ in the indirect pathways becomes negligible in the total noise in $x$. Therefore, in this frequency regime, the direct pathway is dominant and the GNR becomes that of a one-step cascade, which means that it approaches a constant value, independent of frequency. For smaller frequencies, the behavior of the feed-forward motif depends on the relation between $J_{xs}$, $J_{xv}$, $J_{vx}$ and $J_{vv} = -\mu_v$, which are the coupling constants of the direct and indirect pathway and the degradation rate of $V$, respectively (Eq. 3.4). When $|J_{xs}| \ll |J_{uv}|/J_{vv}$, then $\omega \ll \mu_v$, the signal is transmitted more strongly via the indirect pathway than via the direct pathway, in which case the feed-forward motif resembles a two-step cascade (Figs. 3.1c, 3.2c, 3.3c, black solid line); clearly, in the limit that $J_{xs}$ reaches zero, the feed-forward motif becomes a two-step cascade for all frequencies, in which case the the GNR (Eq. 3.10) is constant up to a cut-off frequency $\omega_c^2 = \mu_v (\mu_v + J_{xv})$, falling off as $\omega^{-2}$ for frequencies much larger than that [107]. When on the other hand $|J_{xs}| \gg |J_{uv}|/J_{vv}$, then the direct pathway dominates the gain for all frequencies (Figs. 3.1c, 3.2c, 3.3c, red solid line). Indeed, in terms of the gain, the feed-forward network effectively becomes a one-step cascade. However, the noise via the indirect pathway still contributes to the total noise and therefore this pathway effectively acts as a noise source. This has interesting consequences for the gain-to-noise ratio, as we describe in the next paragraph, since this allows any feed-forward motif to function as either a high-pass or a low-pass filter for information.

The gain-to-noise ratio (Eq. 3.14) of a feed-forward motif varies monotonically with frequency, but it can either be a decreasing or increasing function of $\omega$. Indeed, both a coherent and an incoherent feed-forward motif can either act as a high-pass or low-pass filter for information. We can determine whether the GNR increases or decreases monotonically with frequency, by comparing the GNR at $\omega \to \infty$ to that at $\omega = 0$ (see Eq. 3.14). The GNR is monotonically increasing, meaning that the network acts as a high-pass filter for information, if

$$\langle |\eta_v|^2 \rangle > \langle |\eta_x|^2 \rangle \left( \frac{J_{xs}^2}{J_{xv}} + \frac{2\mu_v J_{xs}}{J_{xs} J_{xv}} \right).$$

(3.15)

The frequency of the inflection point is

$$\omega_{\text{ip}} = \sqrt{\frac{J_{xv}^2 \langle |\eta_v|^2 \rangle + J_{vv}^2 \langle |\eta_x|^2 \rangle}{3 \langle |\eta_x|^2 \rangle}}.$$  

(3.16)

In the next two sections, we will discuss these conditions in more detail for the coherent and incoherent feed-forward motif, respectively.

The data processing inequality states that information that is lost cannot be recovered. Consequently, increasing the length of a cascade reduces information transmission. For this reason, for equal total production rate of the components within a cascade, the GNR of a one-step cascade is always larger than that of a feed-forward motif. The gain by itself
can be larger in a feed-forward motif than in a one-step cascade (Fig. 3.2c, dashed and solid red line). However, the intermediate component V in the indirect pathway introduces an additional noise source, which is not present in the one-step cascade. The coherence term in the gain is not large enough to compensate for this increase in the noise.

We have now specified some general characteristics. In the following we study the coherent and incoherent motif separately.

The coherent feed-forward motif

We will first compare the cff-motif, both the OR and AND types, with two-step cascades. Next we will compare the two motifs with each other. But first, we will start with a couple of observations which apply both to the cff and the ocf. Unless specified otherwise, we assume equal degradation rates for the respective components in the respective motifs and cascades.

The steady-state gain is determined by the average copy number $X (= \partial(x)/\partial(s))$. As a result, if the production of $x$ in a cff equals the production of $x$ in a two-step simple cascade, the gain at zero frequency ($\omega = 0$) is equal. Next, in the linear-noise approximation assumed here, the intrinsic noise arising from the production and degradation events of $X$, $N_x(\omega)$, is equal for the cff and the two-step cascade if the production rates of $x$ are equal (see Eq. 3.12), since we assume throughout that the degradation rates are the same. If the production of $v$ is equal as well the noise $N_v(\omega)$ is equal in the cff and the two-step cascade. However, the transmitted noise $N_{v\rightarrow x}(\omega)$, and thus the total noise $N(\omega)$ can be different, as discussed in more detail below. With respect to the GNR, we observe that for large $\omega$, the cff always has a larger GNR than the two-step cascade, while for small $\omega$, no general results can be presented. Comparing to the one-step cascade, the GNR of the cff is always smaller than that of a one-step cascade if the total production in both networks is equal. This is because $\eta_v$ acts as an additional noise source that corrupts the signal (Figs. 3.1c,3.2c, bottom).

The GNR of a coherent feed-forward motif either increases monotonically with frequency or decreases monotonically with frequency, as mentioned in the previous section. If Eq. 3.15 is satisfied, it increases monotonically, and the motif acts as a high-pass filter for information. We can intuitively understand the terms in Eq. 3.15 as follows. A decrease in the ratio $J_{vS}/J_{xS}$ means that the input signal $s$ is relayed more to $x$ directly than to $x$ via $V$. However, while the direct pathway $S \rightarrow X$ contributes to information transmission at all frequencies — its GNR is flat — the indirect pathway $S \rightarrow V \rightarrow X$ only contributes at low frequencies — the GNR of a two-step cascade falls off as $\omega^{-2}$ for high frequencies. The effect of the indirect pathway, both the gain and the noise, at high frequencies becomes negligible. Indeed, in the limit that $J_{vS}/J_{xS}$ reaches zero, the signal is transmitted completely via the direct pathway only; yet, while the GNR of the direct pathway, a one-step cascade, is flat, the GNR of the cff increases with frequency, because the indirect pathway still adds noise to the signal, especially in the low frequency regime. The presence of the second term on the right-hand side of Eq. 3.15 can be understood by noting that it arises from the interplay between the noise (Eq. 3.12) and the coherence term in the gain (Eq. 3.9). We can understand the dependence on $\mu_v = -J_{vv}$ by noting that at high frequencies $\omega \gg \mu_v$ the coherence and the noise coming from $v$ hardly contribute to the gain and the total noise $N(\omega)$ respectively, while at $\omega = 0$ the coherence decreases with increasing degradation rate as $\mu_v^{-1}$. 
(Eq. 3.9) while the noise coming from $v$ decreases as $\mu_v^{-2}$ (Eq. 3.12). Similarly, at $\omega = 0$, the coherence increases with $J_{xv}$, while the noise coming from $v$ increases with $J_{xv}^2$. Thus at low frequencies decreasing the degradation rate $\mu_v$ and/or increasing the coupling between $V$ to $X$, $J_{xv}$, increases the noise more than it does the gain, thus reducing the GNR at low frequencies, while at high frequencies the influence on the GNR is negligible.

We now discuss the ocff, which combines the two pathways at $X$ according to OR logic. We first compare the ocff with a two-step cascade on the footing of equal production costs for each of the components separately. For $\omega > 0$, the gain of the ocff is always larger than the gain of the two-step cascade (Table. 3.2); for $0 < \omega < \mu_v$, the gain of the ocff is boosted by the coherent interaction between the signals propagated via the direct and indirect pathway, while for $\omega > \mu_v$, the signal is attenuated in the two-step cascade by the finite lifetime of $V$ whereas it can still be propagated in the ocff via the direct pathway. The noise $N(\omega)$ in the ocff motif is smaller than that in the two-step cascade for all $\omega$. The intrinsic noise $N_x(\omega)$ is equal in both networks. However, the extrinsic noise coming from $v$, $g_{xv}^2(\omega)N_v(\omega)$ (see Eq. 3.12) is not. Since the intrinsic noise in $v$, $N_v(\omega)$, is the same in both networks because the production of $v$ is equal, this means that the difference must lie in how this noise is propagated to $x$, which is given by $g_{xv}^2(\omega) = J_{xv}^2/(\omega^2 + J_{xx}^2)$; since $J_{xx}$ is the same, this means that the coupling between $V$ and $X$ in the ocff motif, $J_{xv}^{\text{eff}}$, must be less than that in the two-step cascade, $J_{xv}^{\text{is}}$. Indeed, in the ocff the production of $x$ depends on both $s$ and $v$, while in the two-step cascade it only depends on $v$. Together with the constraint that the production rates of $X$ are equal, this indeed implies that $J_{xv}^{\text{eff}} < J_{xv}^{\text{is}}$.

It can be shown for the ocff motif that for equal production of the species separately in the network, the plateau value of the GNR in the high-frequency regime is higher for the high-pass filter than for the low-pass filter. Therefore, if the goal is to transmit signals reliably at high frequencies, then a suitable parameter set can be chosen that yields a high-pass filter with a high GNR at high frequencies, irrespective of the GNR behavior at low frequencies (Fig. 3.1d, red dashed line). For different constraints on the production, for example constraining the production of only $x$, or constraining the total production of all components, the analysis is more difficult. But, as discussed above, for large $\omega$, the ocff always has a larger GNR than a two-step cascade. The results are summarized in Tables. 3.1.3.2.

In the next paragraphs we study the acff motif. The acff motif combines the two paths at $X$ according to AND-logic and is described by Eq. 3.20. The linearized system has an identical structure to the ocff, and therefore the results are qualitatively similar to those of the ocff. However, quantitatively the results can be different.

First we compare the acff with the corresponding two-step cascade. For equal production of $v$ and $x$ individually the gain of the acff (Eq. A3.29) is always larger than the gain of the two-step cascade (Table. 3.3). This again is due to the coherent interaction between the
direct and indirect pathway at low frequencies, $\omega \ll \mu_v, \mu_x$, while for high frequencies, $\omega \gg \omega_{I}, \omega_{D}$, the gain of a two-step cascade falls more rapidly with frequency than the gain of the direct pathway. Interestingly, the mathematical dependence of the total noise $N(\omega)$ on the network parameters $(J_{I}, \mu_{I}, \ldots)$ of the afc is the same as that of the two-step cascade. Since $N_v(\omega)$ and $N_x(\omega)$ are equal in both networks, this implies that the noise propagated from $v$ to $x$ ($N_{v\rightarrow x} (\omega)$) is equal:

\[
N_{v\rightarrow x, AND} = \frac{\beta^2 (s^2) \langle |\eta_v|^2 \rangle}{(\omega^2 + \mu_v^2) (\omega^2 + \mu_x^2)},
\]

\[
N_{v\rightarrow x, TS} = \frac{k^2 \langle |\eta_v|^2 \rangle}{(\omega^2 + \mu_v^2) (\omega^2 + \mu_x^2)}.
\]
The total rate of production of $x$ by $v$ in the two-step cascade is $k_x \langle v \rangle$ (Eq. A3.7), while, in the linear-noise approximation used here, the average total rate of production of $x$ by $s$ and $v$ in the acff motif is $\beta(\langle s \rangle \langle v \rangle)$ (Eq. A3.28); the latter means that the coupling of $x$ to $v$ is given by $J_{xv} = \beta(s)$. For equal rate of production of $x$ in the two-step cascade and the acff $k_x \langle v \rangle = \beta(\langle s \rangle \langle v \rangle)$ the coupling strengths $J_{xv} = k_x = \beta(s)$ in the two networks are the same, meaning that the extrinsic noise coming from $v$, $N_{v \rightarrow x}$ is indeed the same. Since the gain is larger, but the noise is the same, the GNR of the acff is always larger than that of a two-step cascade.

Again, we can choose different constraints for the production. Let’s constrain the total production of both $v$ and $x$ together to be the same in the two-step cascade and the acff, but we do not require equal production of $v$ and $x$ in both networks separately. For large $\omega$ the GNR of the acff is always larger than that of the two-step cascade. With this constraint this is not necessarily true for small $\omega$. At low frequencies the two-step cascade can have a larger GNR. Intuitively, this is possible if one of the two steps in the two-step cascade is fast, such that it effectively reduces to a one-step cascade at small frequencies, or if the indirect pathway acts as a noise source and is strongly coupled to the response $X$ (see Eq. A3.34).

The acff can not transmit information better at small frequencies than a one-step cascade, given equal total production. For equal production of $x$ — thus at the cost of producing more $V$ — the acff can perform better than the one-step cascade (Eq. A3.32). This is in contrast to the ocff, which also under this constraint has a smaller GNR. The results are summarized in Tables. 3.3.3.4.

Finally, we compare the two motifs ocff and acff for equal separate production of $V$ and $X$. The acff has a larger gain, because it has a steeper response function at equal production. Not only the gain, but also the intrinsic noise ($N_{v \rightarrow x}$) is larger in the acff. These terms have an opposite effect on the GNR. For $\omega \rightarrow \infty$ the acff has a larger GNR, since the noise fluctuations from $v$ are averaged out and only the gain remains important. For small $\omega$, however, no general conclusion for the GNR can be drawn, but a few observations can be made. An increase in the coupling $J_{xv}$ of $S \rightarrow X$ (increasing $\beta$) leads to a larger GNR for the ocff than for the acff. In this case, the direct coupling between $S$ and $X$ dominates in the ocff, whereas this connection is not directly present in the acff due to the AND-logic integration strategy. The ocff ‘averages’ the effects of the direct and indirect pathway, while for the acff-gate the pathways are more connected (Fig. 3.2d). Indeed, one necessary requirement for the ocff to have a larger GNR than the acff at low frequencies is that the direct pathway dominates the output. Another necessary requirement for this is that the GNR of the acff has to have high-pass characteristics (Eqs. A3.48,A3.49).

The incoherent feed-forward motif

The last class of feed-forward loops that we will consider is the incoherent feed-forward (iff) motif (Fig. 3.3a). We will discuss only two different types of iff motifs: one in which negative regulation is implemented in the direct pathway and one in which it is present in the indirect pathway; in the latter case, we always take the negative interaction onto $X$, even though we could also have chosen the alternative in which $S$ acts negatively onto $V$ (and $V$ acts positively on $X$). It is known that the precise topology can influence the noise behavior [38, 111]; however, the qualitative behavior of the GNR of the different possible architectures
3.3 Results

Figure 3.2: The AND coherent feed-forward loop. a) In the acf, both S and V are required to produce X. b) The Langevin equations for the acf. c) The GNR of the acf for different weighings (changing $\alpha$) of the two pathways for equal total production and equal degradation rates. First, for large $\omega$ the direct pathway is dominant and both gain and noise (Eqs. A3.29, A3.30) scale with $\omega^{-2}$, leading to a constant GNR, which is larger than that of a two-step cascade. If $\alpha$ is small (red solid), the production of $V$, $p_v$, is small. To compensate for the small production of $V$, the production of $x$ should be large, leading to a large $\beta$. Note that $\alpha\beta \neq 1$. The gain $g_{\omega \to x}$ scales with $\beta^2\alpha^2$, while the noise $N_{\omega \to x}$ scales as $\beta\alpha$. For small $\omega$, therefore the GNR scales as $\alpha$, while for $\omega \to \infty$, $N_{\omega \to x}$ is averaged out and GNR scales as $\alpha \beta^2$. The dependence of the gain and the noise on $\mu_v$ is slightly different. Therefore, a small bandwidth exists for which the gain decreases more slowly than the noise for increasing $\omega$ (red solid). For large $\alpha$ the opposite reasoning holds. The red dashed line shows the acf with equal production of $V$ and $x$ individually, as the two-step cascade. Both the gain, noise and GNR are larger. It is interesting to note that for small frequencies and equal total production, the acf can have a smaller gain-to-noise ratio than the simple two-step cascade (red solid and gray solid). This is the case if $N_{\omega \to x}$ becomes dominant. Parameters: $k_s=100$, $\mu_v=10$, $\mu_x=100$, $k_{\omega}^1=55$, $k_{\omega}^2=100$, $k_{\omega}^3=5$, $\alpha=0.5, 5.50$ respectively red solid, red dashed, black solid. $\beta$ such that the total production is equal, respectively $\beta=10^{-9.1, 0.01}$. $\mu_s$ sets the timescale. d) The acf (black) for large $\omega$ always has a larger GNR than the acf (red solid and dashed). However, for small $\omega$, depending on kinetic rates, the GNR of the acf can be larger than the acf (red solid). For this to occur, in the acf the direct pathway $S \to X$ should dominate the indirect $S \to V \to X$ pathway. Then, for small $\omega$ the acf acts as an one-step cascade. For the acf this is not the case, because the coupling between $S$ and $V$ is more complex. Parameters: $k_s=100$, $\mu_v=10$, $\mu_x=100$, $J_{S \to V}^{\mu_s}=J_{S \to X}^{\mu_s}=10$, $\beta=1$, $J_{S \to V}^{\omega}=16.67$ and $J_{S \to X}^{\omega}=83.33$ respectively solid and dashed. $\mu_s$ sets the timescale.

is similar to that of the networks studied here. For a more detailed discussion, we refer to App. 3.4.5.

An inspection of the gain in Eq. 3.9 reveals that the coherence term is negative in both types of incoherent feed-forward motifs, since either $J_{S \to S}$ or the product $J_{S \to V} J_{S \to S}$ is negative, respectively. This leads to a reduction of the gain on timescales that are smaller than the
response times of V and X (ω < min {µv, µx}). For ω < µv, the two pathways are exactly out of phase, i.e. the phase difference is −π, while as ω increases, the phase difference reduces to −π/2 (Eq. 3.16).

We model the incoherent regulation using a repressive Hill function (Eq. 3.23b), which is a commonly used coarse-grained description of protein interactions or gene or enzyme regulation. If the direct pathway is repressive, the repression strength depends on the ratio ⟨s⟩/K, with K being the value of s at which X is reduced to half its maximal value. In the limit that K ≫ ⟨s⟩, the repression is very weak, and the influence of the direct pathway is negligible. The iff then effectively reduces to a two-step cascade. In the opposite limit, repression is very strong and the iff becomes adaptive [101]. This means that ⟨x⟩ does not depend on ⟨s⟩. Indeed,

\[
\langle x \rangle = \frac{\nu}{\mu_x} \frac{K \langle v \rangle}{K + \langle s \rangle} = \frac{\nu \alpha}{\mu_x \mu_v} \frac{K \langle s \rangle}{K + \langle s \rangle} \approx \frac{K \nu \alpha}{\mu_x \mu_v}.
\]

(3.21)

In terms of the frequency response, adaptation to constant signals corresponds to a zero-gain at zero frequency, \(g^2(\omega = 0) = 0\)

\[
\lim_{K \ll \langle s \rangle} g^2(\omega = 0) = \frac{(J_{\text{os}} J_{\text{xv}} - J_{\text{uv}} J_{\text{vs}})^2}{\mu_v^2 \mu_x^2 K}
\]

\[
= \frac{K^2 \langle s \rangle^2}{(K + \langle s \rangle)^2} \frac{(\alpha \nu)^2}{\mu_v^2 \mu_x^2} \left[ 1 - \frac{\langle s \rangle}{K + \langle s \rangle} \right]^2 \approx 0.
\]

(3.22)

For large frequencies, only the direct pathway, which in this example is repressive, transmits information. For information transmission it is not important whether the pathway acts negatively or positively on X. The variations in s still affect the variations in x, but with an opposing sign. For the motif where the repression occurs in the indirect pathway, similar conclusions hold, but now the motif functions as an one-step cascade in the case that repression is weak.

An incoherent feed-forward motif with a strong negative interaction in one of the two pathways acts as a high-pass filter for information. This is because of the destructive interference of the two pathways at small frequencies, ω ≪ µv, µx. For higher frequencies, the gain increases because the phase difference between the two pathways decreases and also because the indirect pathway becomes less important as the finite lifetime of V increasingly averages out the variations in S. The gain therefore has high-pass characteristics. Since the noise (Eq. 3.12) is not affected by the destructive interference and has low-pass characteristics, the GNR is high-pass (Fig. 3.3c, red line).

For the incoherent motif, a low-pass GNR is observed only if the negative regulation is small (e.g. K ≫ ⟨s⟩) and the direct pathway is negligible compared to the indirect pathway. Then the motif for small ω resembles a two-step cascade (Fig. 3.3c, black solid line), which indeed exhibits a low-pass GNR. For large ω the direct pathway will dominate, which means that in contrast to a two-step cascade, the GNR reaches a constant as a function of frequency for ω ≫ µv, µx.

Finally, we compare, for completeness, the iff-motif to a two-step cascade. For equal
production of $v$ and $x$ separately, the iff has a lower GNR for small $\omega$ than the two-step cascade (Table 3.6). This is because of the destructive interference between the direct and indirect pathway in the iff, which reduces the gain at low frequencies.

\begin{align}
\frac{dv}{dt} &= \alpha s - \mu v v + \eta_v (t), \quad (3.23a) \\
\frac{dx}{dt} &= -\mu x x + \eta_x (t) + \begin{cases} \\
\frac{\nu K v}{K + s}, & \text{repressive direct} \\
\frac{\nu K s}{K + v}, & \text{repressive indirect} \\
\end{cases} \quad (3.23b)
\end{align}

\textbf{Figure 3.3:} The incoherent feed-forward motif. \textbf{a)} The incoherent motif. Left: The pathway $S\rightarrow V\rightarrow X$ is positive, while the pathway $S\rightarrow X$ is negative. \textbf{b)} The Langevin description of the network \textbf{c)} The coherence terms acts destructively on small timescales, reducing the gain, but not the noise, so that the GNR is reduced. The gain-to-noise ratio of the incoherent motif with a repressive direct link $S\rightarrow X$ has different characteristics, for three different values of $K$ (Eq. 3.23). If $K(\omega)>0$ (black solid) the negative regulation by $S$ diminishes and the iff motif resembles a two-step cascade. For large $\omega$, the indirect pathway is averaged out; at these frequencies one-step characteristics are observed. If $K(\omega)<0$ (red solid), the iff becomes purely adaptive, leading to $g^2=0$ for $\omega=0$. Note that the lines in the noise plot are overlapping, gray dashed: one-step cascade, gray solid: two-step cascade. Parameters: $k_s=100$, $\alpha=1$, $\mu_v=1$, $\mu_x=1$ and $\nu=110.20.11$ for $K(\omega)=0.1, 1, 10$. $\mu_s$ set the timescale.

### 3.3.3 Multimerization

In this section we examine multimerization of intermediate signaling components. In this motif, an intermediate component $v$ is activated by the input signal $s$, which then cooperatively activates the output component $x$ (Fig. 3.4a). The intermediate components could form a protein complex that then binds and activates the output, but it need not be: the intermediate component could also bind the output component, which could be a gene promoter or an enzyme, separately but cooperatively, thereby activating it. This is a common motif in gene regulation and also enzyme regulation.
This system is described by

\[
\frac{dv}{dt} = \alpha_v s(t) - \mu_v v(t) + \eta_v (t), \quad (3.24)
\]

\[
\frac{dx}{dt} = \gamma_v \frac{v^n(t)}{K^n + v^n(t)} - \mu_x x(t) + \eta_x (t)
\]

\[
\approx \gamma_n v^n(t) - \mu_x x(t) + \eta_x (t). \quad (3.26)
\]

Here, \( n \) is a measure for the cooperativity, the number of V molecules that are required to activate X. We assume that the concentration \( v \) is very low, \( K \gg v \), in which case Eq. 3.25 reduces to Eq. 3.26, with \( \gamma_n \approx \gamma_v / K^n \). For this network, the concentration of X and the coupling between \( v \) and \( x \), \( J_{xv} \), depends on the degree of cooperativity \( n \). The concentration \( \langle v \rangle \) is given by \( \gamma_n \langle v^n \rangle / \mu_x \). In the linear-noise approximation this concentration is given by \( \langle v \rangle \approx \gamma_n \langle v^n \rangle / \mu_x \), and the coupling \( J_{xv,n} = n \gamma_n \langle v \rangle^{n-1} \), where the subscript \( n \) in \( J_{xv,n} \) indicates that we consider the coupling between \( v \) and \( x \) when the degree of cooperativity is \( n \). Since we compare the networks on the footing of equal productions costs and the degradation rates are kept constant, we find that the coupling constant \( J_{xv,n} \) for a system in which \( n \) V molecules are required to activate X is related to the coupling constant \( J_{xv,n=1} \) for a system in which only one V molecule is required to activate X, simply via \( J_{xv,n} = nJ_{xv,n=1} \).

For this motif, the gain is given by

\[
g^2(\omega) = \frac{n^2 J_{xv,n=1}^2 v^2}{(\omega^2 + \mu_x^2)(\omega^2 + \mu_v^2)}. \quad (3.27)
\]

It is seen that the gain increases with the cooperativity \( n \).

The noise for this motif is given by

\[
N(\omega) = \frac{N_{x \rightarrow \omega}(\omega)}{N_{v \rightarrow \omega}(\omega)} = \frac{n^2 J_{xv,n=1}^2 \langle |\eta_v|^2 \rangle}{(\omega^2 + \mu_x^2)(\omega^2 + \mu_v^2)} + \frac{\langle |\eta_x|^2 \rangle}{(\omega^2 + \mu_x^2)} \quad (3.28)
\]

\[
= g^2_{v \rightarrow x}(\omega) N_v(\omega) + \frac{\langle |\eta_x|^2 \rangle}{(\omega^2 + \mu_x^2)}. \quad (3.29)
\]

Clearly, increasing \( n \) increases the extrinsic noise in \( x \) that originates in \( v \), \( N_{v \rightarrow x}(\omega) = g^2_{v \rightarrow x} N_v(\omega) \) but not the intrinsic noise in \( x \), \( N_x(\omega) \). Increasing the coupling \( J_{xv,n} = nJ_{xv,n=1} \) does not change the intrinsic noise, but it does affect how fluctuations in \( v \) are amplified at the level of \( x \).

The GNR then reads

\[
\text{GNR} = \frac{J_{xv,n}^2 J_{xv,n=1}}{J_{xv,n=1}^2 \langle |\eta_v|^2 \rangle + \frac{1}{n^2} \langle |\eta_v|^2 \rangle (\omega^2 + \mu_v^2) \langle |\eta_x|^2 \rangle}. \quad (3.30)
\]
From Eq. 3.30 it is clear that the GNR increases with the cooperativity n. This is because while the overall gain $g_{s \to x}$ and and the extrinsic noise $N_{v \to x}(\omega)$ both increase with n, the intrinsic noise $N_x(\omega)$ does not. Interestingly, not only the amplitude of the GNR increases, but also the knee frequency and thus the bandwidth for reliable information transmission. Indeed, the knee frequency $\omega_c$ is set by $\omega_c^2 = \mu^2 + n^2 \sum_{n=1}^{J} \frac{\langle |\eta_n|^2 \rangle}{\langle |\eta_x|^2 \rangle} = \mu v (\mu v + n^2 \gamma_{n=1})$, showing that it increases with n (Fig. 3.4b).

To summarize, we observe that information transmission can be increased by cooperatively activating the output. This could either be achieved via homomultimerization of the intermediate component, or by separate binding of the intermediate molecules to the output component. We note that an increase in n increases the non-linearity of our system and therefore the approximation might break down. However, for the parameters used here (Fig. 3.4b), numerical simulations of the non-linear system agree very well with the linear theory (see App. 3.A.7).

### 3.3.4 Diamond motif

The multimerization motif discussed above could be considered to be a special case of a diamond motif, in which the intermediate components are identical. Here, we consider the general scheme in which they are different: the diamond motif [13, 102, 105, 112, 113]. We will compare the GNR of this motif to that discussed above. Moreover, we will compare the performance of this motif to that of a two-step cascade; if the GNR of the diamond motif is higher than that of a two-step cascade, then this indicates that from the perspective of information transmission it is beneficial to split the signal after the input and recombine the signals downstream, at the output.

We will consider a diamond motif in which the two intermediate components U and W can either form a homodimer $U_2$ or $W_2$, respectively, or a heterodimer $UW$; note that in this network the intermediate components effectively activate the output via AND logic. We will compare the performance of this motif to that of a homodimer motif in which there is only one intermediate component V, which forms a homodimer $V_2$; this corresponds to the scenario discussed before with $n = 2$. The diamond motif is described by

$$\frac{du}{dt} = \alpha s(t) - \mu_u u(t) + \eta_u(t), \quad (3.31)$$
$$\frac{dw}{dt} = \beta s(t) - \mu_w w(t) + \eta_w(t), \quad$$
$$\frac{dx}{dt} = \gamma_u u^2(t) + \gamma_w w^2(t) + 2 \gamma_{uw} uv(t) w(t) - \mu_x x(t) + \eta_x(t).$$

The factor 2 is introduced so that the diamond motif reduces to the motif with only one intermediate component V when the properties of the components U and W are identical and equal to those of V. If $\gamma_u = \gamma_w = 0$, only heterodimer activation is possible. In general, the values of the $\gamma$’s might be different depending on the respective binding kinetics of the components U and W.
The gain of the diamond motif of Eq. 3.31 is

\[
g^2(\omega) = \frac{g_{x \rightarrow w \rightarrow x}^2}{(\omega^2 + \mu_x^2)(\omega^2 + \mu_x^2)} + \frac{g_{x \rightarrow u \rightarrow x}^2}{(\omega^2 + \mu_x^2)(\omega^2 + \mu_x^2)} + \frac{2J_{xu}J_{uw}J_{ux}J_{uw} \left( \omega^2 + \mu_u \mu_w \right)}{N^2 \left( \omega^2 + \mu_x^2 \right) \left( \omega^2 + \mu_x^2 \right)} \tag{3.32}
\]

Here, \( J_{us} = \alpha, J_{ws} = \beta, J_{fu} = 2\gamma_u(u) + 2\gamma_u(w), J_{fx} = 2\gamma_w(w) + 2\gamma_u(w) \). The first two terms in Eq. 3.32 describe the gain due to the transmission of the input signal via the pathways containing \( U \) and \( W \), respectively, while the third term describes the coherence of their interaction at the output \( X \). It can be verified that when \( U \) and \( W \) are identical and equal to \( V \), meaning that \( \alpha = \beta \) and \( \alpha_v = 2\alpha \) and \( J_{us} = J_{ws} = J_{us}/2 \) and \( \langle v \rangle = \langle u \rangle + \langle w \rangle \), \( \gamma_{uw} = \gamma_u = \gamma_w = \gamma_v = \gamma \) and \( J_{fx} = J_{ux} = J_{ux,n=2} = 2\gamma(\langle v \rangle) \), and \( \mu_u = \mu_w = \mu_v \), the gain of the diamond motif equals that of the homodimer motif.

The noise of the diamond motif of Eq. 3.31 is

\[
N(\omega) = \frac{N_{u \rightarrow x}(\omega)}{\langle \eta_u(\omega) \rangle} + \frac{N_{w \rightarrow x}(\omega)}{\langle \eta_w(\omega) \rangle} + \frac{N_x(\omega)}{\langle \eta_x(\omega) \rangle} \tag{3.33}
\]

where we have exploited that \( \langle \eta_u(\omega)\eta_v(-\omega) \rangle = 0 \) when \( U \) and \( W \) are different. If the properties of \( U \) and \( W \) are identical and equal to \( V \), then \( J_{fx} = J_{ux} = J_{ux,n=2} = \mu_u = \mu_v, \langle \eta_u(\omega) \rangle = \langle \eta_w(\omega) \rangle = \langle \eta_x(\omega) \rangle \rangle / 2 \) and the noise of the diamond motif is indeed equal to that of the homodimer motif.

The gain-to-noise ratio for the diamond motif is

\[
g^2 = \frac{\langle J_{xu}J_{wu} \rangle^2 H(\omega, \mu_u) + \langle J_{xf}J_{wu} \rangle^2 H(\omega, \mu_u) + 2\langle J_{xu}J_{wu}J_{xf}J_{wu} \rangle \left( \omega^2 + \mu_u \mu_w \right)}{H(\omega, \mu_u) J_{xu}^2 \left( \langle \eta_u \rangle^2 \right) + H(\omega, \mu_u) J_{xf}^2 \left( \langle \eta_w \rangle^2 \right) + H(\omega, \mu_u) H(\omega, \mu_u) \langle \eta_x \rangle^2} \tag{3.34}
\]

where \( H(x, y) = x^2 + y^2 \).

We now compare the GNR of the diamond motif to that of the homodimer motif. To compare on equal footing, we will assume in what follows below that the production rate of \( x \) is equal in the two motifs \( -\gamma_x(\langle v \rangle)^2 = 2\gamma_{uw}(u) \langle w \rangle + \gamma_u \langle u \rangle^2 + \gamma_w \langle w \rangle^2 \) — and that the production rate of \( v \) in the homodimer motif equals the sum of that of \( u \) and \( w \) in the diamond motif; \( \alpha_u(s) = \alpha(s) + \beta(s) \). If the degradation rates of the intermediate components in the two motifs are equal \(-\mu_u = \mu_u = \mu_w \) — and the coupling between the intermediate components and the output is the same \(-\gamma_{uw} = \gamma_u = \gamma_w \) — then the GNR
of the diamond motif is equal to that of the homodimer motif as it should, since there is no distinction between the components. If the components \( U \) and \( W \) are different, leading, for example, to different couplings \( (\gamma_u \neq \gamma_w) \), still assuming equal degradation rates), the homodimer motif has a larger GNR than the diamond motif at small \( \omega < \mu_u, \mu_w, \mu_v \). In this case, the gain is equal for both processes because the concentration of \( X \) is taken to be the same in both networks. Hence, the difference in the GNR originates from the noise. The noise term \( N_X(\omega) \) is equal in both networks, because the production and degradation rates of \( X \) are taken to be the same in the two networks. However, the extrinsic noise propagated from the intermediate components is larger in the diamond motif, \( N_{u \rightarrow x} + N_{w \rightarrow x} > N_{v \rightarrow x} \). It can be shown that under the constraints that a) the production of \( v \) equals the total production of \( u \) and \( w \) and b) the production of \( x \) is the same in both motifs, the extrinsic noise is minimized when the coupling of \( u \) and \( w \) to \( x \) are identical.

We stress, however, that the gain-to-noise ratio of this diamond motif is higher than that of a simple two-step cascade, with one intermediate component \( V \) that does not activate \( X \) in a cooperative manner. Indeed, for a diamond motif the coherent interaction between the two pathways plays a crucial role. While for a motif with non-cooperative activation of the output by the two pathways, this coherence between the two pathways exactly compensates for the decrease of the gain of each independent pathway, for a network with cooperative interaction, the coherence term increases the GNR over that of a simple two step cascade. In other words, splitting the input signal into two and then recombining them with AND logic at the output does increase the gain-to-noise ratio. Finally, we study a motif in which only the heterodimer \( UW \), and not the homodimers \( U_2 \) and \( W_2 \), can activate the output \( X \). We thus consider the case that \( \gamma_w = \gamma_u = 0 \), and consider what happens if the degradation rates of \( U \) and \( W \) are allowed to be different. With unequal degradation rates, a bandpass filter for information is possible, if the coupling of one pathway to \( x \) is stronger than the coupling of the other pathway. We take \( \mu_w > \mu_u \), such that the pathway \( S \rightarrow W \rightarrow X \) is capable of transmitting information on faster timescales than the pathway \( S \rightarrow U \rightarrow X \). If \( \beta \gg \alpha \), the input signal is relayed more strongly via the pathway containing \( W \), and the other pathway acts as a noise source. Consequently, for small frequencies, \( \omega < \mu_u \), signal transmission will be corrupted by noise originating at \( u \), but for \( \mu_u < \omega < \mu_w \) this noise is averaged out. We thus obtain a band-pass filter for information transmission (Fig. 3.4c, red solid). By actively changing the degradation rates \( \mu_u \) and \( \mu_w \), the cell can tune the frequency range of the band.

3.4 Discussion

Our analysis reveals that feed-forward motifs and diamond motifs are very rich information processing devices. More specifically, our study shows that both coherent and incoherent feed-forward motifs can either act as low- or high-pass filters for information. This behavior can be understood by noting that while at high frequencies the direct pathway always dominates the output signal, at low frequencies the contribution of each pathway to the output varies between networks, depending on the coupling constants between the components in the network; moreover, at low frequencies, the output strongly depends on the nature of the
Figure 3.4: The diamond motif. a) The diamond motif combines two pathways (S→V→X and S→W→X), originating from the same source at the response X. With homodimerization U and W correspond to two molecules of the species V. b) The difference between the gain, noise and GNR for a network with different levels of cooperativity (Eq. 3.24). For increasing cooperativity (n increases), the gain and the noise both increase. The increase in the noise is smaller than in the gain, since N_X is unaffected by the cooperative interactions and therefore the GNR increases. Next, also the knee frequency, and thus the bandwidth, increases, since the GNR scales with \( \omega^{-2} \) for \( \omega^2 > n^2 \mu_v \). Parameters: \( k_s=100, k_v=10, k_b=10, \mu_v=100, \mu_x=1 \), \( \mu_s \) sets the timescale. c) The GNR for the diamond motif where U and W have different degradation rates \( \mu_u < \mu_w \). If \( J_{us} > J_{uw} \) the GNR is low-pass (black solid), since on long timescales \( t > \mu_u^{-1} \), the signal is transmitted while on shorter timescales most transmission is corrupted by the intrinsic noise. If \( J_{us} < J_{uw} \) (red solid line), the slow signal variations transmitted via w are corrupted by noise from u. For faster variations, the noise from u is averaged out and the signal can be transmitted with larger reliability. Note that the gain for all three parameter sets is equal, and the lines thus overlap. The bandpass characteristic for the GNR is thus due to the different dependence of the noise on \( \omega \). Parameters: \( k_s=100, \mu_x=10 \), two-step cascade: \( \mu_v=10, k_{uv}^s=500, k_{vw}^s=100, \alpha \) to equalize production of v, \( \gamma_v \) to equalize production of x, diamond motif: \( \mu_u=1, \mu_w=100, \gamma_u=\gamma_w=0, \gamma_{uw} \) to equalize production of x; \( \beta \) to equalize \( \langle v^2 \rangle = \langle u^2 \rangle + \langle w^2 \rangle \), \( \alpha = 0.05k_{uv}^s0.5k_{bw}^b, 0.95k_{tv}^s \), respectively red solid, red dashed, black solid. \( \mu_x \) sets the timescale.

interaction between the two pathways. If the interaction is coherent, as in coherent feed-forward motifs, then the frequency dependence of the gain, noise and gain-to-noise ratio tends to have low-pass characteristics. If, however, not only at high frequencies, but also at low frequencies the direct pathway dominates the response, then the gain-to-noise ratio...
can have high-pass characteristics; in this case, the signal is predominantly transmitted via the direct pathway, while the indirect pathway acts as a noise source, masking this signal at low frequencies. For an incoherent feed-forward motif, the gain is low at low frequencies because of the destructive interference between the two pathways; consequently, the frequency dependence of the gain-to-noise ratio of incoherent feed-forward motifs tends to have high-pass characteristics. However, an incoherent motif can also act as a low pass filter for information. This scenario arises when the direct pathway acts weakly on the output at low frequencies; then at low frequencies the output is dominated by the indirect pathway, which exhibits low-pass signal filtering, while at high frequencies it is dominated by the direct pathway. More generally, our analysis demonstrates that by changing the coupling constants between the components the frequency-dependence of the gain, noise and gain-to-noise ratio can be sculpted in almost any desirable manner.

For equal total production cost of all molecules in the network a coherent feed-forward motif has a lower information transmission capacity than a simple one-step motif. However, if we allow for a higher production cost in the feed-forward motif (e.g. we require equal production of \( x \), but allow for the additional production of the intermediate component \( V \)), interestingly a coherent feed-forward motif in which the two pathways are combined following AND logic can have a larger GNR than the one-step cascade. Combining the pathways following OR logic has always a smaller GNR than the one-step cascade, even for larger total production. This demonstrates for these simple cascades a possible advantage of coincidence detection.

Our results also underscore the important observation that the power spectrum of the output signal is not a good measure for information transmission [107]. The results on the incoherent feed-forward motif provide a concrete illustration of this idea: while the frequency dependence of the gain exhibits band-pass characteristics, the frequency dependence of the gain-to-noise ratio shows high-pass characteristics; indeed, at high frequencies not only the gain, and hence the output, decreases, but also the noise. The coherent feed-forward motif with a high-pass gain-to-noise ratio provides another striking example: while the gain and hence the output decreases with frequency, the gain-to-noise ratio increases; this is because the high gain at low frequencies is masked by the high noise. Our results thus show that in order to draw any conclusion on how reliably a network can transmit time-varying signals, one needs to measure not only the power spectrum of the output \( P_{xx}(\omega) \), but also the power spectrum of the input \( P_{ss}(\omega) \) and their cross-power spectrum \( P_{sx}(\omega) \): from these quantities one can obtain the gain \( g^2(\omega) \equiv |P_{sx}(\omega)|^2/P_{sx}^2(\omega) \) and the frequency dependence noise \( N(\omega) \), and hence the gain-to-noise ratio (see Eq. 3.7 and Eq. 3.8).

Finally, our analysis of the diamond motifs reveals that cooperatively activating the output can markedly enhance the gain, as well as the gain-to-noise ratio. The latter is due to coincidence detection: While variations in the input signal lead to correlated variations in the intermediate components that tend to boost the output, noise generates uncorrelated fluctuations in the intermediate components, which couple less strongly to the output. We emphasize that this mechanism is very generic. Indeed, cooperative activation of the output can be implemented in many ways: via the formation of homo- or heteromultimers that then bind and activate the output component, or via the individual binding of the inter-
mediate components to the output component. While cooperative activation of the output via one and the same type of intermediate component, as in the case of homodimerization, increases the overall gain-to-noise ratio, cooperative output activation via components that are different, as in the case of heterodimerization of the intermediate components, makes it possible to mold the frequency dependence of the gain-to-noise ratio, even allowing for band-pass filters for information.

3.5 Acknowledgements

I would like to thank Philippe Nghe for a critical reading of this chapter.
3.A Supplementary Information

All cascades have the following simple (linear) birth-death process for the signal

\[
\frac{ds}{dt} = k_s - m_s s + \eta_s(t). \quad \text{(A3.1)}
\]

3.A.1 Simple cascades

Simple cascades, cascades without feed-forward interaction, are described using roman symbols for the kinetic rates, while motifs with feed-forward interactions, are described using Greek symbols. The network for the one-step simple cascade is described by

\[
\frac{dx}{dt} = k_x s - m_x x + \eta_x(t). \quad \text{(A3.2)}
\]

Gain, noise and gain-to-noise are

\[
g^2(\omega) = \frac{k_x^2}{\omega^2 + m_x^2},
\]

\[
N(\omega) = \frac{\langle |\eta_x|^2 \rangle}{\omega^2 + m_x^2},
\]

\[
g^2(\omega) \quad N(\omega) = \frac{k_x^2}{2 \langle s \rangle} \cdot \quad \text{(A3.3-3.5)}
\]

The network for the two-step simple cascade is described by

\[
\frac{dv}{dt} = k_v s - m_v v + \eta_v(t),
\]

\[
\frac{dx}{dt} = k_x v - m_x x + \eta_x(t). \quad \text{(A3.6-3.7)}
\]

Gain, noise and gain-to-noise are

\[
g^2(\omega) = \frac{(k_v k_x)^2}{(\omega^2 + m_v^2)(\omega^2 + m_x^2)}, \quad \text{(A3.8)}
\]

\[
N(\omega) = \frac{k_x^2 \langle |\eta_v|^2 \rangle}{(\omega^2 + m_v^2)(\omega^2 + m_x^2)} + \frac{\langle |\eta_x|^2 \rangle}{(\omega^2 + m_x^2)}, \quad \text{(A3.9)}
\]

\[
g^2(\omega) \quad N(\omega) = \frac{(k_v k_x)^2}{k^2_x \langle |\eta_v|^2 \rangle + (\omega^2 + m_v^2) \langle |\eta_x|^2 \rangle}, \quad \text{(A3.10)}
\]

Production constraints

In the following sections we will compare the one-step (os) and two-step (ts) cascades with different feed-forward motifs. We always assume that degradation rates for proteins are
equal, unless specified otherwise. We use three different constraints, such that the comparison is performed on an equal footing. These constraints are

1. Equal production of $x$, free production of $v$.

\[ p_X^x = p_X^x, p_X^v = p_X^v. \]  \hspace{1cm} (A3.11)

We note that this also implies $\langle |\eta_x|^2 \rangle = \langle |\eta_x^v|^2 \rangle = \langle |\eta_x^f|^2 \rangle$.

2. Equal total production of $x$ and $v$,

\[ p_X^x + p_X^v = p_X^X + p_X^Y, p_X^v = p_X^v. \]  \hspace{1cm} (A3.12)

3. Equal production of $x$ and $v$ separately (this constraint has no meaning for the one-step cascade)

\[ p_X^x = p_X^x, p_Y^v = p_Y^v. \]  \hspace{1cm} (A3.13)

We note that this also implies $\langle |\eta_x|^2 \rangle = \langle |\eta_x^v|^2 \rangle = \langle |\eta_x^f|^2 \rangle$.

Unless specified otherwise the degradation rates are equal for components in either cascade, e.g. $m_{v} = \mu_v$.

3.4.2 QR coherent feed-forward

The network is described by

\[ \frac{dv}{dt} = \alpha s - \mu_v v + \eta_v(t), \]  \hspace{1cm} (A3.14)

\[ \frac{dx}{dt} = \beta s + \gamma v - \mu_x x + \eta_x(t), \]  \hspace{1cm} (A3.15)

and the gain, noise and gain-to-noise the gain, noise and gain-to-noise (see Eqs. 3.9, 3.12 and 3.14)

\[ g^2(\omega) = \frac{(\alpha \gamma + J_{wx} \beta)^2 + J_{xx}^2 \omega^2}{(\omega^2 + \mu_v^2)(\omega^2 + \mu_x^2)}, \]  \hspace{1cm} (A3.16)

\[ N(\omega) = \gamma^2 \left( \langle |\eta_v|^2 \rangle + (\omega^2 + \mu_v^2) \langle |\eta_x|^2 \rangle \right), \]  \hspace{1cm} (A3.17)

\[ \frac{g^2(\omega)}{N(\omega)} = \frac{(\alpha \gamma + \mu_v \beta)^2 + \beta^2 \omega^2}{\gamma^2 \left( \langle |\eta_v|^2 \rangle + (\omega^2 + \mu_v^2) \langle |\eta_x|^2 \rangle \right)}. \]  \hspace{1cm} (A3.18)

In Fig. 3.10 (left) the dependence of the gain and the phase difference between the direct $S \rightarrow X$ and indirect $S \rightarrow V \rightarrow X$ pathway as a function of frequency is shown. For $\omega < \mu_v$, the two pathways have no phase difference, but for an increase in frequency the phase
<table>
<thead>
<tr>
<th>Cons.</th>
<th>one-step</th>
<th>(GNR) one-step</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.p.x</td>
<td>(k_x = \beta + \frac{\alpha \gamma}{\mu_v})</td>
<td>(g^2 \frac{\langle \eta_x \rangle^2}{N} &gt; GNR^{\text{eff}})</td>
</tr>
<tr>
<td>E.t.p.</td>
<td>Always larger</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>two-step</th>
<th>(GNR) two-step at (\omega = 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.p.x</td>
<td>(k_x k_v = \beta + \frac{\alpha \gamma}{\mu_v})</td>
</tr>
<tr>
<td>E.t.p.</td>
<td>((k_x + \mu_v) k_v = (\alpha + \beta) \mu_v + \gamma \alpha)</td>
</tr>
<tr>
<td>E.s.p.</td>
<td>(k_v = \alpha, \quad k_x = \gamma + \frac{\beta \mu_v}{\alpha})</td>
</tr>
</tbody>
</table>

Table 3.1: The results for the gain, noise and gain-to-noise ratio for the one-step cascade and the two-step cascade in parameters of the ocf, given the constraint conditions. For the two-step only the \(\omega \to 0\) limit is given, since for large \(\omega\) the ocf always has a larger \(GNR\). We assume always \(\mu_v = m_v\). E.p.x: Equal production of x, e.t.p.equal total production, e.s.p.equal separate production.

The difference increases and as result the maximum of the coherent term in the gain decreases. The magnitude of the coherent term also decreases due to the time-averaging over the finite lifetime of the intermediate component V. Even for a constant phase-difference, at large \(\omega\) the gain decreases.

In Table 3.1 and Table 3.2 we list the expression for the \(GNR\) for different production constraints. The \(GNR\) of the one-step cascade given equal production of x is always larger than that of the ocf. Compared to the two-step cascade, for \(\omega \to \infty\) the ocf motif always has a larger \(GNR\), since the noise source \(N_{\omega \to x}\) is averaged out, and only an effective one-step cascade with constant \(GNR\) remains. Thus we only compare the ocf with the two-step cascade for \(\omega \to 0\).

The various constraints lead to different results. The first scenario, where we independently constrain \(p_v\) and \(p_x\) and take \(\mu_v = m_v\) in both cascades leads to an expression for the \(GNR\) in the two-step cascade that is smaller than that for the ocf for all \(\omega\). The gain in both the ocf and the two-step cascade are precisely equal (not shown), and therefore the larger \(GNR\) is due to the increase in the noise \((N(\omega))\) in the two-step cascade. A constraint that allows for more freedom in the network is such that only the production of x is constrained.
but there is free production of $v$. We observe that for $\omega = 0$, the GNR for the ocff is larger than the two-step if

$$\gamma^2 \left\langle |\eta_v|^2 \right\rangle^{\text{ocff}} < \frac{2}{k_v} \left( \mu_v \beta + \alpha \gamma \right)^2$$  \hspace{1cm} (A3.19)\n
$$2\alpha \left\langle s \right\rangle \gamma^2 < \frac{2}{k_v} \left( \mu_v \beta + \alpha \gamma \right)^2,$$  \hspace{1cm} (A3.20)\n
$$\alpha \gamma^2 < \frac{\left( \mu_v \beta + \alpha \gamma \right)^2}{k_v}.\hspace{1cm} (A3.21)$$

This inequality is not satisfied if e.g. $k_v \to \infty$, since then the two-step cascade effectively becomes an one-step cascade and as a result, has a larger GNR than the ocff.

For $\omega \to \infty$ the GNR of the ocff with high-pass filter characteristics can have a larger plateau value than an ocff with low-pass characteristics, under the constraint that the production of $v$ and $x$ separately are equal. This can be shown by solving for the inequality

$$\lim_{\omega \to \infty} \text{GNR}^{\text{hp}} > \lim_{\omega \to \infty} \text{GNR}^{\text{lp}} \Rightarrow \left( \beta_{\text{hp}} \frac{\gamma_{\text{lp}} \alpha_{\text{hp}}}{\mu_{\text{lp}}} \right)^2 > \left( \frac{\beta_{\text{lp}}}{\mu_{\text{lp}}} \right)^2,$$  \hspace{1cm} (A3.22)\n
Low-pass (lp) $\to \beta_{\text{lp}} < \left( \beta_{\text{lp}} + \frac{\gamma_{\text{lp}} \alpha_{\text{lp}}}{\mu_{\text{lp}}} \right) \frac{\alpha_{\text{lp}}}{\beta_{\text{lp}}} + \frac{2 \mu_{\text{lp}}}{\gamma_{\text{lp}}}.\hspace{1cm} (A3.23)\n
High-pass (hp) $\to \beta_{\text{hp}} < \left( \beta_{\text{hp}} + \frac{\gamma_{\text{hp}} \alpha_{\text{hp}}}{\mu_{\text{hp}}} \right) \frac{\alpha_{\text{hp}}}{\beta_{\text{hp}}} + \frac{2 \mu_{\text{hp}}}{\gamma_{\text{hp}}}.\hspace{1cm} (A3.24)\n
The first line describes the inequality. The second line describes the requirement for $\beta_{\text{lp}}$ and the third line the requirement for $\beta_{\text{hp}}$, which are obtained following substitution of $\left\langle |\eta_v|^2 \right\rangle$.

### Table 3.2: Summary of the results for the ocff.

<table>
<thead>
<tr>
<th>Cons.</th>
<th>one-step vs ocff</th>
<th>two-step vs ocff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal production of $x$</td>
<td>$\text{GNR}^{\text{pos}} &gt; \text{GNR}^{\text{ocff}}$</td>
<td>Parameter dependent</td>
</tr>
<tr>
<td>Equal total production</td>
<td>$\text{GNR}^{\text{pos}} &gt; \text{GNR}^{\text{ocff}}$</td>
<td>Parameter dependent</td>
</tr>
<tr>
<td>Equal separate production</td>
<td>$\text{GNR}^{\text{pos}} &lt; \text{GNR}^{\text{ocff}}$</td>
<td></td>
</tr>
</tbody>
</table>
and \( \langle |\eta_x|^2 \rangle \) in Eq. 3.15. From the constraints of equal separate production we obtain

\[
\left\langle \left| \eta^p_x \right|^2 \right\rangle = \left\langle \left| \eta^p_y \right|^2 \right\rangle \rightarrow \alpha^p \equiv \alpha^p,
\]
(3A.25)

\[
\left\langle \left| \eta^b_x \right|^2 \right\rangle = \left\langle \left| \eta^b_y \right|^2 \right\rangle \rightarrow \beta^b + \frac{\gamma^b \alpha^b}{\mu^p} \equiv \beta^b + \frac{\gamma^b \alpha^b}{\mu^p}.
\]
(3A.26)

Solving the system of inequalities Eqs. 3A.22-3A.24, with Eqs. 3A.25,3A.26 using Mathematica, it can be shown that these can always be fulfilled. However, the full expressions are unwieldy to present here. Even if \( \beta^b \rightarrow \infty \), reflecting that the low-pass filter effectively is a one-step cascade, parameters can be found for which the high-pass filter can still have a larger GNR for large \( \omega \). However, the difference between the low-pass and high-pass filter is negligible.

3.A.3 AND coherent feed-forward

The network is described by

\[
\frac{dv}{dt} = \alpha s - \mu v + \eta_v(t),
\]
(3A.27)

\[
\frac{dx}{dt} = \beta v s - \mu x + \eta_x(t).
\]
(3A.28)

and the gain, noise and gain-to-noise (Eq. 3.9, 3.12 and 3.14)

\[
g^2(\omega) = \frac{\beta^2(\nu)^2(\omega^2 + 4\mu^2_v)}{(\omega^2 + \mu^2_v)(\omega^2 + \mu^2_x)}
\]
(3A.29)

\[
N(\omega) = \frac{\beta^2(\nu)^2 \left\langle |\eta_v|^2 \right\rangle + (\omega^2 + \mu^2_v) \left\langle |\eta_x|^2 \right\rangle}{(\omega^2 + \mu^2_x)(\omega^2 + \mu^2_v)}
\]
(3A.30)

\[
\text{GNR}(\omega) = \frac{\beta^2(\nu)^2(\omega^2 + 4\mu^2_v)}{\beta^2(\nu)^2(\omega^2 + 4\mu^2_v) + (\omega^2 + \mu^2_v) \left\langle |\eta_x|^2 \right\rangle}
\]
(3A.31)

For equal production of \( x \) and for \( \omega \rightarrow \infty \), the GNR of a one-step cascade is equal to the GNR of the acff. For \( \omega = 0 \), we obtain the following relation

\[
\text{GNR}^\text{acff}(\omega = 0) < \text{GNR}^\text{casc}(\omega = 0)
\]
(3A.32)

\[
\frac{\beta^2 \left\langle \nu \right\rangle^2}{\left\langle |\eta_x|^2 \right\rangle} < 4 \frac{\beta^2 \left\langle \nu \right\rangle^2}{\left\langle |\eta_v|^2 \right\rangle} + \left( \frac{2 \beta(\nu)}{\mu v} \right)^2 \left\langle \left| \eta_v \right|^2 \right\rangle
\]
(3A.33)

where the last line holds for \( \beta(\nu)/\alpha < 3 \left\langle |\eta_x|^2 \right\rangle / \left\langle |\eta_v|^2 \right\rangle \). This inequality can be sufficed for example in the limit that \( \beta \rightarrow 0 \). Then the acff has a larger GNR than the one-step cascade for \( \omega = 0 \). This limit corresponds to a situation where the noise \( N_{v \rightarrow x} \) (Eq. 3.12)
<table>
<thead>
<tr>
<th>Cons.</th>
<th>one-step</th>
<th>GNR one-step</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.p.x</td>
<td>( k_x = \beta(v) )</td>
<td>( \frac{g^2}{N} = \frac{\beta^2(v)^2}{\langle \eta_x^2 \rangle} )</td>
</tr>
<tr>
<td>E.t.p.</td>
<td>( k_x = \alpha + \beta(v) )</td>
<td>( \frac{g^2}{N} = \frac{(\alpha + \beta(v))^2}{\langle \eta_x^2 \rangle} &gt; \text{GNR}^{\text{acff}} )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>two-step</th>
<th>GNR two-step at ( \omega = 0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.p.x</td>
<td>( k_x k_v = \beta(v^{\text{acff}}) \mu_v )</td>
<td>( \frac{g^2}{N} = \frac{\langle s \rangle \alpha \beta}{\langle \eta_x \rangle} \mu_v^2 + \frac{\langle s \rangle \alpha \beta^2}{k_x^2} \langle \eta_v \rangle^2 )</td>
</tr>
<tr>
<td>E.t.p.</td>
<td>( (k_x + \mu_v) k_v = \mu_v \left(\alpha + \beta(v^{\text{acff}})\right) )</td>
<td>( \frac{g^2}{N} = \frac{k_x \alpha (\beta(s) + \mu_v)}{2 \langle s \rangle (k_x + \mu_v)^2} )</td>
</tr>
<tr>
<td>E.s.p.</td>
<td>( k_v = \alpha, )</td>
<td>( \frac{g^2}{N} = \frac{\langle s \rangle \alpha \beta^2}{\mu_v^2 k_v^2} )</td>
</tr>
<tr>
<td></td>
<td>( k_x = \frac{\mu_v}{k_v} \beta(v) )</td>
<td>( \frac{g^2}{N} = \frac{\langle s \rangle \alpha \beta^2}{\mu_x^2 + \langle s \rangle \beta^2 \langle \eta_v \rangle^2} )</td>
</tr>
</tbody>
</table>

Table 3.3: The results for the gain, noise and gain-to-noise ratio for the one-step cascade and the two-step cascade in parameters of the acff, given the constraint conditions. For the two-step only the \( \omega \to 0 \) limit is given, since for large \( \omega \) the ocff always has a larger GNR. E.p.x: Equal production of \( x \), e.t.p.: equal total production, e.s.p.: equal separate production.

<table>
<thead>
<tr>
<th>Cons.</th>
<th>one-step vs acff</th>
<th>two-step vs acff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal production of ( x )</td>
<td>Parameter dependent</td>
<td>Parameter dependent</td>
</tr>
<tr>
<td>Equal total production</td>
<td>( \text{GNR}^{\text{es}} &gt; \text{GNR}^{\text{acff}} )</td>
<td>Parameter dependent</td>
</tr>
<tr>
<td>Equal separate production</td>
<td>( \text{GNR}^{\text{es}} &lt; \text{GNR}^{\text{acff}} )</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4: Summary of the results for the acff.
is negligible, and the noise contributions $N(\omega)$ in both motifs are similar. The gain in the 
acff is larger than in the one-step cascade. This seems contradictory, but is a result of the 
production constraint. In the acff the production of $x$, $p_x$, is $\beta \langle s \rangle \langle v \rangle$, such that if $\langle v \rangle$ is 
very small, the production rate $\beta$ becomes very to ensure equal production of $x$. However 
in the acff, the production of $x$ is $\beta \langle s \rangle + \gamma \langle v \rangle$ and if $\langle v \rangle$ is nearly zero, production still is 
possible through the direct pathway $S \rightarrow X$. Due to this coincidence coupling between the 
two pathways, the gain for the acff is larger than for the one-step cascade.

As expected, the acff has a larger GNR than the simple two-step cascade if we equalize 
both $p_v$ and $p_x$ separately. More interesting is the behavior at low frequencies for the two 
other constraint types. If we equalize the total production the two-step cascade has a larger 
GNR than the acff if

$$
\frac{\mu_v \left( \beta \langle v \rangle + \alpha - k_v \right)}{2 \langle s \rangle (\mu_v + k_x)} > \frac{\beta \langle s \rangle \beta^2 \langle v \rangle^2 (\omega^2 + 4\mu_v^2)}{2 (\mu_v + k_v) \langle |\eta^\text{MD}|^2 \rangle + (\omega^2 + \mu_v^2) \langle |\eta^\text{MD}|^2 \rangle}
$$

(A3.34)

$$
\frac{\beta \langle s \rangle + \mu_v \beta \langle v \text{acff} \rangle + \alpha - k_v}{k_x + \mu_v} > 4,
$$

(A3.35)

where this is possible if $\beta \langle s \rangle \gg k_x$ or $\alpha \gg k_v$. From the constraint condition of total pro-
duction we have the equality $(k_x + \mu_v) k_v = \mu_v \alpha + \beta \alpha \langle s \rangle$. Taken these relations together, 
we observe that for $\beta \langle s \rangle \gg k_x$ we require $k_v \gg 1$, while for $\alpha \gg k_v$ we require to $k_x \gg 1$. 
In both cases the two-step cascade transforms into a one-step cascade because one of the 
two steps directly tracks the changes upstream.

The other option, where we constrain the production of $x$, but not $v$,

$$
k_x = \beta \langle s \rangle \frac{\left\langle v_{\text{acff}} \right\rangle}{\langle v_{\text{two}} \rangle},
$$

(A3.36)

leads to the following expression for the gain-to-noise ratio at low frequencies for the two-
step cascade

$$
\text{GNR} (\omega = 0) = \frac{\left( \mu_v \beta \langle v_{\text{acff}} \rangle \right)^2}{\beta \langle s \rangle \langle v_{\text{acff}} \rangle \left( |\eta^x|^2 \right) + \mu_v^2 \langle |\eta_x|^2 \rangle}.
$$

(A3.37)
The ratio of the the GNR’s is

\[
\frac{\text{GNR}^{\text{eff}}(\omega = 0)}{\text{GNR}^{\text{ss}}(\omega = 0)} = 4 \left( \frac{\beta (s) \langle v^{\text{eff}} \rangle^2}{\langle v^{\text{ss}} \rangle^2} \right)^2 \frac{\langle |\eta_x|^2 \rangle}{\langle |\eta_x|^2 \rangle} + \mu_v^2 \langle v^{\text{ss}} \rangle^2 \langle |\eta_x|^2 \rangle = 4 \left( \frac{\beta (s) \langle v^{\text{eff}} \rangle^2}{\langle v^{\text{ss}} \rangle^2} \right)^2 \frac{\langle |\eta_x|^2 \rangle}{\langle |\eta_x|^2 \rangle} + \mu_v^2 \langle v^{\text{ss}} \rangle^2 \langle |\eta_x|^2 \rangle \tag{A3.38}
\]

\[
= 4 \left( \frac{\alpha^2(s) k_v}{\mu_v^2} + \frac{\mu_v^2 \langle v^{\text{ss}} \rangle^2 \langle |\eta_x|^2 \rangle}{2(s)(\beta(s))^2} \right) \tag{A3.39}
\]

\[
= 4 \frac{\alpha + F}{k_v + F}, \quad \text{where} \quad F = \frac{k_v \mu_v^2 \langle \eta_x^2 \rangle}{2\alpha \beta^2 (s)}. \tag{A3.40}
\]

If \( k_v \gg \alpha \) the two-step cascade has a larger GNR. This reflects again a situation where the two-step cascade has one very fast step and acts effectively as a single one-step cascade.

3.4.4 Comparison of the coherent feed-forward AND and OR motifs

We study if the GNR of the acff is larger or smaller than the GNR of the ocff. We equalize both production of \( v \) and \( x \), such that we have

\[
\begin{align*}
p^{\text{ocff}}_v &= p^{\text{acff}}_v \rightarrow \alpha^{\text{ocff}} = \alpha^{\text{acff}} = \alpha, \tag{A3.41} \\
p^{\text{ocff}}_x &= p^{\text{acff}}_x \rightarrow \beta^{\text{ocff}} = \beta^{\text{acff}}(v), \tag{A3.42}
\end{align*}
\]

and using these constraints and Eq. A3.18, A3.31, we obtain the ratio

\[
\frac{\text{GNR}^{\text{acff}}}{\text{GNR}^{\text{ocff}}} = \frac{\left( \beta^{\text{ocff}} + \frac{\alpha \gamma}{\mu_v} \right)^2 (\omega^2 + 4\mu_v^2)}{\left( \beta^{\text{acff}} + \frac{\alpha \gamma}{\mu_v} \right)^2 (\omega^2 + \mu_v^2)} \times \frac{\gamma^2 \langle |\eta_x|^2 \rangle + (\omega^2 + \mu_v^2) \langle |\eta_x|^2 \rangle}{\left( \beta^{\text{acff}} + \gamma \right)^2 \langle |\eta_x|^2 \rangle + (\omega^2 + \mu_v^2) \langle |\eta_x|^2 \rangle}. \tag{A3.43}
\]

In the limit \( \omega \rightarrow \infty \) we obtain

\[
\frac{\text{GNR}^{\text{acff}}}{\text{GNR}^{\text{ocff}}} = \frac{\left( \beta^{\text{ocff}} + \frac{\alpha \gamma}{\mu_v} \right)^2}{\left( \beta^{\text{acff}} \right)^2} > 1, \tag{A3.45}
\]
such that for large $\omega$ the acff has a larger GNR. In the other limit $\omega = 0$, we obtain

$$
\frac{\text{GNR}_{\text{acff}}}{\text{GNR}_{\text{ocff}}} = \frac{(\beta_{\text{eff}} + \frac{\alpha \gamma}{\mu v})^2}{2 (\beta_{\text{eff}}^2 \mu_v^2 + (\alpha \gamma)^2 + 2\alpha \beta \gamma \mu_v^2) \left( \frac{\beta_{\text{eff}} \mu_v}{\alpha} + \gamma \right)^2} \frac{\gamma^2 \left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2}{\left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2},
$$

(A3.46)

$$
= 4 \frac{\gamma^2 \left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2}{\left( \frac{\beta_{\text{eff}} \mu_v}{\alpha} + \gamma \right)^2}\frac{\left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2}{\left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2},
$$

(A3.47)

such that if

$$
4 \left( \gamma^2 \left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2 \right) < \left( \frac{\beta_{\text{eff}} \mu_v}{\alpha} + \gamma \right)^2 \left( \left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2 \right),
$$

the ocff has a larger GNR. This condition (Eq. A3.48) can be satisfied if in the ocff motif the direct pathway $S \rightarrow X$ couples (large $\beta$) much stronger to $X$ than the indirect pathway $S \rightarrow V \rightarrow X$ (small $\alpha$). Interestingly, the parameter values for which this condition is satisfied, result in an GNR for the acff motif with a high-pass filter. This we show by comparing the two extrema for the acff ($\omega \rightarrow \infty$ and $\omega = 0$).

$$
\text{GNR}_{\text{acff}}^{\text{HD}} (\omega = 0) = \frac{4 \mu_v^2 (\beta_{\text{eff}} + \frac{\alpha \gamma}{\mu v})^2}{\left( \frac{\beta_{\text{eff}} \mu_v}{\alpha} + \gamma \right)^2}\frac{\left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2}{\left| \eta_v \right|^2 + \mu_v^2 \left| \eta_x \right|^2},
$$

(A3.49)

$$
\lim_{\omega \rightarrow \infty} \text{GNR}_{\text{acff}}^{\text{HD}} (\omega) = \frac{(\beta_{\text{eff}} + \frac{\alpha \gamma}{\mu v})^2}{\left| \eta_x \right|^2},
$$

(A3.50)

where it can be shown that, given the condition in Eq. A3.48 is satisfied, the acff has a larger GNR for large frequencies than for small frequencies.
3.6.5 Incoherent feed-forward motif

The network is described by

\[ \frac{dv}{dt} = \alpha s - \mu_v v + \eta_v(t), \tag{A3.51} \]
\[ \frac{dx}{dt} = \frac{\nu K v}{K + s} - \mu_x x + \eta_x(t). \tag{A3.52} \]

The gain, noise and gain-to-noise (Eqs. 3.9, 3.12 and 3.14) in general terms are

\[ g^2(\omega) = \frac{\left(J_{vx} J_{xx} + J_{vx} J_{xx}\right)^2 + J_{vx}^2 \omega^2}{\left(\omega^2 + \mu_v^2\right) \left(\omega^2 + \mu_x^2\right)}, \tag{A3.53} \]
\[ N(\omega) = \frac{J_{xx}^2 \left(\eta_v\right)^2 + \left(\omega^2 + \mu_x^2\right) \left(\eta_x\right)^2}{\left(\omega^2 + \mu_v^2\right) \left(\omega^2 + \mu_x^2\right)}, \tag{A3.54} \]
\[ g^2(\omega) = \frac{\left(J_{vx} J_{xx} + J_{vx} J_{xx}\right)^2 + J_{vx}^2 \omega^2}{\left(\omega^2 + \mu_v^2\right) \left(\omega^2 + \mu_x^2\right)}, \tag{A3.55} \]

First, we study the influence of the topology. For the incoherent feed-forward, four different topologies exist with respect to the regulation. It is known that different topologies have influence on the noise characteristics. Here we show that the GNR indeed depends on the specific topology, comparing both a different position for the negative regulation, and the type of integration of the two pathways at the X component.

Instead of negatively regulating X by V or S, we can also negatively regulate V by S, such that we have

\[ \frac{dv}{dt} = \frac{\alpha K v}{K + x} - \mu_v v + \eta_v(t), \tag{A3.56} \]
\[ \frac{dx}{dt} = \beta s + \gamma v - \mu_x x + \eta_x(t), \tag{A3.57} \]

where we have assumed the signals combine at X following OR strategy. In the previous section, we equalized production and studied the difference in the coupling parameters $J_{ij}$. Here, instead of equalizing production, we equalize the Jacobian coefficients (as a result the gain is equal). For these coupling parameters, we compare the production of the components (and thus the noise terms). We refer to the topology with superscript $V$, for negative
regulation of $V$, and superscript $X$ for negative regulation of $X$.

\[
\left| J_{V_{VS}}^V \right| = J_{V_{VS}}^X \to \alpha^V \frac{K_V}{(K_V + \langle s \rangle)^2} = \alpha^X, \quad (A.3.58)
\]
\[
J_{V_{SX}}^V = J_{V_{XS}}^X \to \beta^V = \nu X \frac{K \langle v \rangle}{(K + \langle s \rangle)^2}, \quad (A.3.59)
\]
\[
J_{V_{SV}}^V = J_{V_{VS}}^X \to \gamma^V = \nu X \frac{K}{K + \langle s \rangle}. \quad (A.3.60)
\]

And following these equalities, we write for the noise terms

\[
\eta_X^V = 2 \alpha^V \frac{K_V}{K_V + S} = 2 (K_V + \langle s \rangle) \alpha^X > \eta_X^X, \quad (A.3.61)
\]
\[
\eta_X^X = 2 (\beta \langle v \rangle + \gamma \langle v \rangle) = 2 \nu X \frac{K \langle v \rangle}{K + \langle s \rangle} \left( 1 + \frac{\langle s \rangle}{K + \langle s \rangle} \right) > \eta_X^X. \quad (A.3.62)
\]

For equal coupling constants the noise terms (or equivalently the production terms) in the iff motif are larger for negative regulation on $V$ than on $X$.

If the pathways combine following AND logic we have

\[
\frac{dx}{dt} = \gamma_{VS} - \mu_x x + \eta_x(t). \quad (A.3.63)
\]

Now, we equalize production terms

\[
p_V^V = \alpha^V \frac{K_V}{K_V + \langle s \rangle}, \quad p_X^V = \alpha^X \langle s \rangle, \quad (A.3.64)
\]
\[
p_V^X = \gamma \langle v \rangle \langle s \rangle, \quad p_X^X = \nu \frac{K \langle v \rangle}{K + \langle s \rangle}, \quad (A.3.65)
\]

and obtain $\alpha^X = \alpha^V K_V (K_V + \langle s \rangle)^{-1}$ and $\gamma = \nu K (K + \langle s \rangle)^{-1}$. We then compare the Jacobian terms

\[
\left| J_{V_{VS}}^V \right| = \alpha^V \frac{K_V}{(K_V + \langle s \rangle)^2}; \quad J_{V_{XS}}^X = \alpha^X = \alpha^V \frac{K_V}{(K_V + \langle s \rangle) \langle s \rangle}, \quad (A.3.66)
\]
\[
J_{V_{SX}}^V = \gamma \langle v \rangle = \nu \frac{K \langle v \rangle}{\langle s \rangle + K}; \quad J_{V_{XS}}^X = \nu \frac{K \langle v \rangle}{\langle s \rangle + K}^{2}, \quad (A.3.67)
\]
\[
J_{V_{SV}}^V = \gamma \langle s \rangle = \nu \frac{K}{\langle s \rangle + K}; \quad J_{V_{SV}}^X = \nu \frac{K}{\langle s \rangle + K}^{2}. \quad (A.3.68)
\]

and we observe that $J_{V_{XS}}^X > \left| J_{V_{VS}}^V \right|$, while $J_{V_{SX}}^V > J_{V_{XS}}^X$. Therefore we conclude that at large frequencies the AND integration with negative regulation on $V$ has a larger GNR than for negative regulation on $X$. For small frequencies the GNR depends on the specific parameters.

Compared with a one-step cascade for equal production of $x$ (so $v$ is not constrained),
we have for the GNR of the incoherent motif

\[ p_x^I = p_x^{10} \rightarrow \frac{\nu K (v)}{K + \langle s \rangle} = k_x^{10} (s) \rightarrow \frac{\nu K}{K + \langle s \rangle} = \frac{k_x^{10} \mu_x}{\alpha}, \quad (A.39) \]

leading to

\[ g^2 \frac{N}{N} = \frac{(k_x^{10} \mu_x \alpha)^2}{(k_x^{10} \alpha)^2} \left[ \frac{K}{K + \langle s \rangle} \right]^2 + \frac{(k_x^{10} \alpha)^2}{(k_x^{10} \alpha)^2} \frac{\omega^2}{\langle |\eta_x|^2 \rangle} < \frac{k_x^{10}}{\alpha}, \quad (A.37) \]

such that the incoherent motif always has a smaller GNR than the one-step motif for equal production of \( x \).

Compared to the two-step motif and equal total production we distinguish two scenarios. First, we assume equal production of \( v \) and obtain

\[ p_x^{12} = p_x^{20} \rightarrow \frac{\nu K}{K + \langle s \rangle} = \frac{k_x \mu_v \alpha}{\alpha}, \quad (A.31) \]

and for the GNR of the iff cascade

\[ g^2 \frac{N}{N} = \frac{(k_x \mu_v \alpha)^2}{(k_x \alpha)^2} \left[ \frac{K}{K + \langle s \rangle} \right]^2 + \frac{(k_x \alpha)^2}{(k_x \alpha)^2} \frac{\omega^2}{\langle |\eta_x|^2 \rangle} \right] \quad (A.32) \]

Compared to the GNR of a two-step cascade, for small \( \omega \) the GNR of the iff is smaller due to the negative interference in the iff, while for large \( \omega \) this is larger than the GNR of a two-step cascade due to the direct pathway.

In the other scenario only require \( p_x^{12} = p_x^{20} \), to obtain

\[ g^2 \frac{N}{N} = \frac{(k_x \mu_v \alpha)^2}{2 \left( k_x \alpha \right)^2} \left[ \frac{K}{K + \langle s \rangle} \right]^2 + \frac{(k_x \alpha)^2}{(k_x \alpha)^2} \frac{\omega^2}{\langle |\eta_x|^2 \rangle} \right] \quad (A.33) \]

Due to the direct pathway the gain-to-noise ratio for the iff is larger for \( \omega \rightarrow \infty \). For \( \omega = 0 \)
Table 3.6: Summary of the results for the comparison between the iff and the two-step cascade.

we obtain

\[
\begin{align*}
\text{iff} & \quad \frac{q^2 (\omega = 0)}{N (\omega = 0)} > \frac{(k_v k_x) \alpha}{(k_v k_x)^2} \left( \frac{K}{K + s} \right)^2 \left( \frac{1}{s} \right) \langle s \rangle + \mu^2 \left( \langle \eta_x \rangle^2 \right) \\
\text{simple 2 step} & \quad \frac{q^2 (\omega = 0)}{N (\omega = 0)} > \frac{(k_v k_x)^2}{2 \left( k_x k_v \langle s \rangle \right) + \mu^2 \left( \langle \eta_x \rangle^2 \right) + \frac{\mu^2}{(k_x k_v)^2} \langle \eta_x \rangle^2} \\
\end{align*}
\]

where the above inequality is valid in the following two scenario’s: 1) if \( k_v < \alpha \), the iff motif has a larger GNR, since the signal is transmitted to \( v \) with larger gain than in the two-step cascade. 2) If \( K \gg \langle s \rangle \), the negative feedback is greatly suppressed and we observe a general two-step cascade instead of an iff motif. If the first condition is not satisfied, the two-step simple cascade has a larger GNR for \( \omega = 0 \). The second condition is required if the difference in the gain due to the first condition is not large enough.

3.6 Multimerization

The network for multimerization is described by

\[
\begin{align*}
\frac{dv}{dt} &= \alpha s - \mu_v v + \eta_v (t), \\
\frac{dx}{dt} &= \gamma_v v^n - \mu_x x + \eta_x (t),
\end{align*}
\]  

where \( n \) is the number of proteins that jointly activate \( X \). The gain, noise and gain-to-
noise

\[
g^2(\omega) = \frac{n^2k_s^2(1-n) \alpha^2n\gamma_0^2}{\mu_s^2(1-n)^2(\omega^2 + \mu_s^2 + \mu_v^2)(\omega^2 + \mu_v^2 + \mu_c^2)}.
\]

(A3.79)

\[
N(\omega) = \frac{n^2k_s^2(1-n) \alpha^2n\gamma_0^2}{\mu_s^2(1-n)^2(\omega^2 + \mu_s^2 + \mu_v^2)(\omega^2 + \mu_v^2 + \mu_c^2)} \left( \eta_v(\omega) \right)^2 + \mu_s^2(1-n)^2(\omega^2 + \mu_v^2 + \mu_c^2) \left( \eta_x(\omega) \right)^2.
\]

(A3.80)

\[
GNR(\omega) = \frac{n^2k_s^2(1-n) \alpha^2n\gamma_0^2}{\mu_s^2(1-n)^2(\omega^2 + \mu_s^2 + \mu_v^2)(\omega^2 + \mu_v^2 + \mu_c^2)} \left( \eta_v(\omega) \right)^2 + \mu_s^2(1-n)^2(\omega^2 + \mu_v^2 + \mu_c^2) \left( \eta_x(\omega) \right)^2.
\]

(A3.81)

For equal production of \(x \) (\(v \) is by construction in all cascades equal), we have

\[
\gamma_v, u = \gamma_{n-1} = \frac{n-1}{k_s^2} \mu_v^{n-1},
\]

(A3.82)

which after we substitute this in Eq. A3.81, results in Eq. 3.30.

The diamond motif is described by

\[
\frac{du}{dt} = \alpha u(t) - \mu_u u(t) + \eta_u(t),
\]

(A3.83)

\[
\frac{dw}{dt} = \beta u(t) - \mu_w w(t) + \eta_w(t),
\]

\[
\frac{dx}{dt} = \gamma_u u^2(t) + \gamma_w w^2(t) + 2\gamma_{uw} u(t) w(t) - \mu_x x(t) + \eta_x(t).
\]

For \(\mu_u = \mu_v = \mu_w, \alpha = C_I\beta \) and \(\gamma_u = \gamma_{uw} = C_{II}\gamma_w \), where we have introduced the coefficients \(C_I \) and \(C_{II} \) to study form of the gain, noise and GNR for differences between the intermediates \(U \) and \(W \) in a general context, the expression for the gain, noise and gain-to-noise are

\[
g^2(\omega) = \frac{4k_s^2\beta^4 (1 + 2C_I + C_{II}^2)^2 \gamma_u^2}{\mu_s^2 \mu_v^2 \mu_c^2 \mu_w H(\mu_v, \omega) H(\mu_w, \omega)}.
\]

(A3.84)

\[
N(\omega) = \frac{4k_s^2\beta^4 \gamma_u^2}{\mu_s^2 \mu_v^2 \mu_c^2 \mu_w H(\mu_v, \omega) H(\mu_w, \omega)} \left( A^2 \left[ \eta_u(\omega) \right]^2 + (1 + C_I)^2 \left[ \eta_w(\omega) \right]^2 + \mu_s^2 \mu_v^2 \mu_w H(\mu_w, \omega) \left[ \eta_x(\omega) \right]^2 \right).
\]

(A3.85)

\[
GNR(\omega) = \frac{4k_s^2\beta^4 (1 + 2C_I + C_{II}^2)^2 \gamma_u^2}{4k_s^2\beta^2 \gamma_u^2} \left( A^2 \left[ \eta_u(\omega) \right]^2 + (1 + C_I)^2 \left[ \eta_w(\omega) \right]^2 + \mu_s^2 \mu_v^2 \mu_w H(\mu_w, \omega) \left[ \eta_x(\omega) \right]^2 \right).
\]

(A3.86)
where \( A = (1 + C_I C_{II}) \) and \( H(x, y) = x^2 + y^2 \).

We take \( C_{II} = 1 \) and compare this cascade to a cascade with only homodimerization of the component \( U (\beta = \gamma_w = \gamma_{uw} = 0) \). For readability, we refer to this cascade, as if it has an intermediate component \( V \), and if required, subscripts denote \( U, V \) and \( W \).

Equal production at the intermediate level \( p_V = p_U + p_W \) and at the level of \( X (p_X^{dm} = p_X^{homo}) \) gives

\[
(1 + C_I) \beta = \alpha_v \tag{A3.77}
\]

\[
\gamma_{uw} = \gamma_v \frac{(v)^2}{U^2 + 2 \langle u \rangle \langle w \rangle + \langle w \rangle^2} = \gamma_v \frac{(u) + (w))^2}{U^2 + 2 \langle u \rangle \langle w \rangle + \langle W \rangle^2} \\
= \gamma_v \frac{1}{\alpha_u + \beta_w} = \gamma_v. \tag{A3.78}
\]

Inserting these equalities in Eq. A3.84, we observe that the gain is equal to Eq. A3.79 for \( n = 2 \),

\[
g^2 (\omega) = \frac{4k^2 s^2}{\mu_s^2 \mu_0^2 (\omega^2 + \mu_s^2) (\omega^2 + \mu_0^2)} \\
= \frac{4k^2 (1 + C_I)^4 \beta^4}{\mu_s^2 \mu_0^2 (\omega^2 + \mu_s^2) (\omega^2 + \mu_0^2)}, \tag{A3.89}
\]

and the noise is

\[
N(\omega) = \frac{4k^2 (1 + C_I)^4 \beta^2 \gamma_{uw}^2 \left( \langle |\eta_u|^2 \rangle + \langle |\eta_v|^2 \rangle \right) + \mu_s^2 \mu_0^2 (\omega^2 + \mu_s^2) \langle |\eta_e|^2 \rangle}{\mu_s^2 \mu_0^2 (\omega^2 + \mu_s^2) (\omega^2 + \mu_0^2)}. \tag{A3.90}
\]

where Eq. A3.92 is exactly Eq. A3.85. Therefore, unequal production of \( u \) and \( w \) is not important, as long as the sum is constrained (and all \( \gamma \)'s are equal).

Next we compare to a two-step cascade. We first assume the diamond motif to be completely symmetric for the intermediate components \( U, W, \alpha = \beta, \mu_u = \mu_w \) and \( \gamma_u = \gamma_w = \gamma_{uw} \). We constrain the individual production of each step in the cascade

\[
p_{uv}^{two} = p_{uw}^{dm} + p_{uw}^{dm} \rightarrow k_v = 2\alpha_v \tag{A3.93}
\]

\[
p_{ux}^{two} = p_{ux}^{dm} \rightarrow k_xk_v = 4\gamma \frac{\alpha^2 (s)}{\mu_v}. \tag{A3.94}
\]

In the limit \( \omega \rightarrow \infty \) the diamond motif has a larger GNR independent of any kinetic
rates, since

\[ \frac{GNR_{dm}}{GNR_{two}} = \frac{4\mu_w^2}{\mu_v^2} = 4. \]  \hspace{1cm} (A3.95)

In the opposite limit, \( \omega \to 0 \), we have

\[ \frac{GNR_{dm}}{GNR_{two}} = \frac{(8 \langle s \rangle \alpha \gamma_{uw} + 4\mu_w^2)}{(8 \langle s \rangle \alpha \gamma_{uw} + \langle s \rangle \mu_v^2)} > 1. \]  \hspace{1cm} (A3.96)

Thus the symmetric diamond motif has a larger GNR for \( \omega \to 0 \) and \( \omega \to \infty \). For equal \( \mu_v = m_v \), the GNR of the diamond motif is larger for all frequencies.

The above case is for \( \alpha = \beta \). Here we use \( \alpha = C_I \beta \), but \( \mu_v = \mu_w = m_v \). The ratio of the GNR’s for \( \omega \to \infty \) is simply 4 (Eq. A3.95). For the limit \( \omega \to 0 \) we have

\[ \frac{GNR_{dm}}{GNR_{two}} = \frac{4 \left( (1 + C_I) \langle s \rangle \alpha \gamma_{uw} + \mu_w^2 \right)}{4 \left( (1 + C_I) \langle s \rangle \alpha \gamma_{uw} + \mu_v^2 \right)} > 1. \]  \hspace{1cm} (A3.97)

We do observe that the more asymmetric the diamond motif becomes, the ratio of the GNR’s decreases. The performance of the diamond motif then becomes more similar to the two-step cascade.

More interestingly is to have different degradation rates \( \mu_v = C_{III} \mu_w \), where \( C_{III} \) is an arbitrary constant. The equal production constraints then result in the following two expressions

\[ p_{w}^{two} = p_{v}^{dm} + p_{w}^{dm} \rightarrow k_v = 2\alpha, p_{x}^{two} = p_{x}^{dm} \rightarrow k_xk_v = m_v \gamma \frac{\alpha^2 \langle s \rangle}{C_{III} \mu_w^2}. \]  \hspace{1cm} (A3.98)

For high frequencies the ratio of the GNR of the two-step and diamond motif is

\[ \frac{GNR_{dm}}{GNR_{two}} = \frac{C_{III}^2 \mu_w^2}{(1 + C_{III})^2 m_v^2}, \]  \hspace{1cm} (A3.99)

which does not lead to a unique conclusion whether which of the two is larger. For \( \omega = 0 \), we have

\[ \frac{GNR_{dm}}{GNR_{two}} = \frac{2 \left( (1 + C_{III}) \langle s \rangle \alpha \gamma_{uw} + 2C_{III}^2 \mu_w^2 \right)}{4 \left( (1 + C_{III}) \langle s \rangle \alpha \gamma_{uw} + C_{III}^2 \mu_w^2 \right)}. \]  \hspace{1cm} (A3.100)

Again, the ratio of the GNR depends on the kinetic rates.

3A.7 Numerical validation

The linearization used in the derivations can change the characteristics of the frequency response, since a linearized system does not change the frequency of the transmitted signal. This may not be the case for a nonlinear system. In this section we show the compari-
son between our analytical results, following the linear noise approximation, for the power spectrum and the result from numerical simulations of the full non-linear network. For the numerical simulations we use the Gillespie algorithm. The negative regulation as present in the incoherent feed-forward motif, or the positive regulation as in the dimerization process are calculated through Hill-like interactions between the components. In the Gillespie simulation we calculated the propensities for every reaction using the coarse grained Hill-expressions for the propensities, such that

\[ V \xrightarrow{r} V + X, \quad (A3.101) \]

where \( r \) is

\[ r = \frac{kK}{S + K}, \quad (A3.102) \]

where here the actual copy number of \( S \) is used, and not \( \langle s \rangle \), as in the linearized expressions (Eq. A3.55).

The power spectra are calculated using \( 2^{10} \) (1024) exponentially distributed frequencies from \( \omega = 10^{-1} \) to \( \omega = 10^2 \) and averaged over 16 neighboring frequencies to obtain a single data point. In total we have 64 data points. Fourier transforms and power spectra are directly integrated during runtime. We simulated a minimum of 27 blocks of 5000s. Results are shown in Figs. 3.5-3.9.

![Figure 3.5: The results (symbols) of the Gillespie simulations for the OR-coherent feed-forward motif. Since this motif is linear, we do not expect any deviations between the numerical simulations and the analytical results. We only show the result for one set of parameters: \( k_6=100, \alpha=11, \beta=1, \gamma=10.1, \mu_\omega=10, \mu_\delta=10, \mu_\xi \) sets the timescale.](image)

3.A.8 Influence of the phase

In Fig. 3.10 we show in some more detail the precise influence of the phase difference between two pathways that combine at a downstream component. A larger phase difference corresponds to a decrease in the gain. However, the phase difference between the pathways is not the only relevant parameter to study the gain. The gain is also greatly reduced if the signal variations are much faster than the lifetime of the components in the cascade. If this is the case, the individual components can not track the variations and start to time-average the variations, thereby losing the specific information in the high frequencies.
Figure 3.6: The results (symbols) of the Gillespie simulations for the AND-coherent feed-forward motif. Although this motif is non-linear, the results of the simulations are in good agreement with those of the linear analysis (continuous lines). In $P_{xx}$ and $\text{Re}[P_{xx}]$ the symbols of the simulations results are on top of the lines in the analytical results. Parameters: a) as in Fig. 3.2c red solid, b) Fig. 3.2c red dashed, c) Fig. 3.2c black solid.

Figure 3.7: The results (symbols) of the Gillespie simulations for the incoherent feed-forward motif. Although this motif is non-linear, the results of the simulations are in good agreement with those of the linear analysis (continuous lines). In $P_{xx}$ and $\text{Re}[P_{xx}]$ the symbols of the simulations results are on top of the lines in the analytical results. Parameters: a) as in Fig. 3.3c red solid, b) Fig. 3.3c red dashed, c) Fig. 3.3c black solid.

Figure 3.8: The results (symbols) of the Gillespie simulations for the dimerization process. Here the motif is non-linear. We model the propensity function following a Hill function, $k(V) = k_{\text{max}} V^n / (V^n + K^n)$. Next, we take $K \gg V$, such that propensity is approximately $k(V) \approx k_{\text{max}} (V/K)^n$ as discussed in the main text. The results of the simulations are in good agreement with those of the linear analysis (continuous lines). In $P_{xx}$ and $\text{Re}[P_{xx}]$ the symbols of the simulations results are on top of the lines in the analytical results. Parameters: a) two-step process ($n=1$), b) dimer process ($n=2$), c) trimer process ($n=3$). Parameters are as in Fig. 3.4b.
3.A Supplementary Information

Figure 3.9: The results (symbols) of the Gillespie simulations for the heterodimerization motif, with $\gamma_u = \gamma_w = 0$. Although this motif is non-linear, the results of the simulations are in good agreement with the results of the linear analysis (continuous lines). In $P_{\infty}$ and $\text{Re}[P_{\infty}]$ the symbols of the simulations results are on top of the lines in the analytical results. Parameters: a) as in Fig. 3.4c red solid, b) Fig. 3.4c red dashed, c) Fig. 3.4c black solid.

Figure 3.10: In these panels we show the gain (black dashed, right axis) and phase (black solid, left axis). In panel a) we show the gain and the phase for a cff-motif. The decrease of the gain corresponds with the increase in the phase difference between the indirect $S \rightarrow V \rightarrow X$ and direct pathway $S \rightarrow X$. In panel b) we show a similar plot, but now for the diamond motif with pathways of equal length, but with different degradation rates of the intermediate components. Again we observe that the initial decrease in the gain coincidences with the increase in phase difference. But although the phase difference decrease for larger $\omega$ again, the gain continues to decrease, due to the time-averaging of the fluctuations over the lifetime of the intermediate components.