A combination of transcriptional and microRNA regulation improves the stability of the relative concentrations of target genes

Supplementary Information

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Contents

1 Models in detail 2
2 Master equation 4
3 Linear noise approximation and simulations: steady state discrepancy 6
4 Comparison between NM4 and micFFL 7
5 Comparison with the explicit promoter dynamics 10
6 Stability analysis 10
7 Steady state analysis with the logic approximation 12
# Models in detail

**Gillespie reactions** In the following we briefly introduce the reactions involved in the circuits considered in the main text that we used to simulate the models through the implementation of Gillespie’s direct algorithm [43]. As discussed in the text, we assume a titrative interaction of the miRNA with both its targets while the interaction between transcription factor (TF) and target (T) is modelled as a Hill function.

Set of reactions for the NM1:

| Reaction | Rate | Picture |
|----------|------|---------|
| * → $m_1$ | $k_{m_1}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $m_1$ → $m_1 + p_1$ | $k_{p_1 m_1}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $p_1$ → $p_1 + m_2$ | $k_{m_2 p_1}^* (p_1)^n + k_{m_2}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $m_2$ → $m_2 + p_2$ | $k_{p_2 m_2}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $m_1$ → $m_1 + p_1$ | $\gamma_{m_1 m_1}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $p_1$ → | $\gamma_{p_1 p_1}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $m_2$ → | $\gamma_{m_2 m_2}^*$ | ![Diagram 1](https://via.placeholder.com/150) |
| $p_2$ → | $\gamma_{p_2 p_2}^*$ | ![Diagram 1](https://via.placeholder.com/150) |

Set of reaction for the NM2:

| Reaction | Rate | Picture |
|----------|------|---------|
| * → $s$ | $k_s^*$ | ![Diagram 2](https://via.placeholder.com/150) |
| * → $m_1$ | $k_{m_1}^*$ | ![Diagram 2](https://via.placeholder.com/150) |
| $m_1$ → $m_1 + p_1$ | $k_{p_1 m_1}^*$ | ![Diagram 2](https://via.placeholder.com/150) |
| $s + m_1$ → $c_1$ | $k_{c_1 s}^* m_1$ | ![Diagram 2](https://via.placeholder.com/150) |
| $c_1$ → $s$ | $\alpha_{c_1 c_1}^* (p_1)^n$ | ![Diagram 2](https://via.placeholder.com/150) |
| $p_1$ → $p_1 + m_2$ | $k_{m_2 p_1}^* (p_1)^n + k_{m_2}^*$ | ![Diagram 2](https://via.placeholder.com/150) |
| $m_2$ → $m_2 + p_2$ | $k_{p_2 m_2}^*$ | ![Diagram 2](https://via.placeholder.com/150) |
| $s$ → | $\gamma_{s s}^* s$ | ![Diagram 2](https://via.placeholder.com/150) |
| $m_1$ → | $\gamma_{m_1 m_1}^* m_1$ | ![Diagram 2](https://via.placeholder.com/150) |
| $c_1$ → | $(1 - \alpha)^* c_1 c_1$ | ![Diagram 2](https://via.placeholder.com/150) |
| $p_1$ → | $\gamma_{p_1 p_1}^* p_1$ | ![Diagram 2](https://via.placeholder.com/150) |
| $m_2$ → | $\gamma_{m_2 m_2}^* m_2$ | ![Diagram 2](https://via.placeholder.com/150) |
| $p_2$ → | $\gamma_{p_2 p_2}^* p_2$ | ![Diagram 2](https://via.placeholder.com/150) |
Set of reaction for the NM3:

| Reaction | Rate | Picture |
|----------|------|---------|
| * → s    | $k_s^*$ | ![Diagram 3](image) |
| * → m_1  | $k_{s \rightarrow m_1}^*$ | |
| m_1 → m_1 + p_1 | $k_{m_1 \rightarrow (m_1 + p_1)}^*$ | |
| p_1 → p_1 + m_2 | $k_{m_1 \rightarrow (p_1 + m_2)}^*$ | |
| m_2 → m_2 + p_2 | $k_{m_2 \rightarrow (m_2 + p_2)}^*$ | |
| s + m_2 → c_2 | $k_{s + m_2 \rightarrow (c_2)}$ | |
| c_2 → s    | $\alpha_{c_2 \rightarrow s}^*$ | |
| s → s      | $\alpha_{s \rightarrow s}^*$ | |
| m_1 → m_1 | $\gamma_{m_1 \rightarrow (m_1)}^*$ | |
| p_1 → p_1 | $\gamma_{p_1 \rightarrow (p_1)}^*$ | |
| m_2 → m_2 | $\gamma_{m_2 \rightarrow (m_2)}^*$ | |
| c_2 → (1 - \alpha) \gamma_{c_2 \rightarrow c_2}^* | | |
| p_2 → $\gamma_{p_2 \rightarrow p_2}^*$ | |

Set of reaction for the maimed NM4:

| Reaction | Rate | Picture |
|----------|------|---------|
| * → s    | $k_s^*$ | ![Diagram 4](image) |
| * → m_1  | $k_{s \rightarrow m_1}^*$ | |
| m_1 → m_1 + p_1 | $k_{m_1 \rightarrow (m_1 + p_1)}^*$ | |
| s + m_1 → c_1 | $k_{s + m_1 \rightarrow (c_1)}$ | |
| c_1 → s    | $\alpha_{c_1 \rightarrow s}^*$ | |
| s → s      | $\alpha_{s \rightarrow s}^*$ | |
| m_2 → m_2 | $\gamma_{m_2 \rightarrow (m_2)}^*$ | |
| s + m_2 → c_2 | $k_{s + m_2 \rightarrow (c_2)}$ | |
| c_2 → s    | $\alpha_{c_2 \rightarrow s}^*$ | |
| s → s      | $\alpha_{s \rightarrow s}^*$ | |
| m_1 → m_1 | $\gamma_{m_1 \rightarrow (m_1)}^*$ | |
| c_1 → (1 - \alpha) \gamma_{c_1 \rightarrow c_1}^* | | |
| p_1 → $\gamma_{p_1 \rightarrow (p_1)}^*$ | |
| m_2 → $\gamma_{m_2 \rightarrow (m_2)}^*$ | |
| c_2 → (1 - \alpha) \gamma_{c_2 \rightarrow c_2}^* | | |
| p_2 → $\gamma_{p_2 \rightarrow p_2}^*$ | |
### Set of reaction for the NM5:

| Reaction | Rate | Picture |
|----------|------|---------|
| * → s₁ | \( k^*_s \) | ![Diagram](5) |
| * → s₂ | \( k^*_s \) |
| * → m₁ | \( k^*_m₁ \) |
| s₁ + m₁ → c₁ | \( k^{on}_{c₁} s₁ · m₁ \) |
| c₁ → s₁ | \( \alpha \gamma_{c₁} c₁ \) |
| m₁ → m₁ + p₁ | \( k^*_m₁ \) |
| p₁ → p₁ + m₂ | \( k^*_m₂ (p₁)^n \) |
| m₂ → m₂ + p₂ | \( k^*_p₂ m₂ \) |
| s₂ + m₂ → c₂ | \( k^{on}_{s₂} m₂ \) |
| c₂ → s₂ | \( \gamma_{s₂} s₂ \) |
| s₁ → m₁ | \( \gamma_{s₁} m₁ \) |
| c₁ → (1 − α) \( \gamma_{c₁} c₁ \) |
| p₁ → \( \gamma_{p₁} p₁ \) |
| m₂ → \( \gamma_{m₂} m₂ \) |
| c₂ → (1 − α) \( \gamma_{c₂} c₂ \) |
| p₂ → \( \gamma_{p₂} p₂ \) |

### Set of reaction for the micFFL:

| Reaction | Rate | Picture |
|----------|------|---------|
| * → s | \( k^*_s \) | ![Diagram](6) |
| * → m₁ | \( k^*_m₁ \) |
| s₁ + m₁ → c₁ | \( k^{on}_{c₁} s₁ · m₁ \) |
| c₁ → s₁ | \( \alpha \gamma_{c₁} c₁ \) |
| m₁ → m₁ + p₁ | \( k^*_m₁ \) |
| p₁ → p₁ + m₂ | \( k^*_m₂ (p₁)^n \) |
| m₂ → m₂ + p₂ | \( k^*_p₂ m₂ \) |
| s₂ + m₂ → c₂ | \( k^{on}_{s₂} m₂ \) |
| c₂ → s₂ | \( \gamma_{s₂} s₂ \) |
| s₁ → m₁ | \( \gamma_{s₁} m₁ \) |
| c₁ → (1 − α) \( \gamma_{c₁} c₁ \) |
| p₁ → \( \gamma_{p₁} p₁ \) |
| m₂ → \( \gamma_{m₂} m₂ \) |
| c₂ → (1 − α) \( \gamma_{c₂} c₂ \) |
| p₂ → \( \gamma_{p₂} p₂ \) |

### 2 Master equation

In the following we report the master equations for the various circuits analyzed, with \( \{s \rightarrow n₁, m₁ \rightarrow n₂, c₁ \rightarrow n₃, p₁ \rightarrow n₄, m₂ \rightarrow n₅, c₂ \rightarrow n₆, p₂ \rightarrow n₇\} \) and the step operator \( e_j^k = \sum_{l=0}^{\infty} \frac{k^l}{l!} \frac{\partial^{n_l}}{\partial n_j^{n_l}} \) as defined in [32]. The rates are rescales as in the main text.
\[
\frac{\partial P(n_1, \tau)}{\partial \tau} = \left\{ k_{s_1} (E_1^{-1} - 1) + \gamma_{s_1} (E_1^1 - 1) n_1 k_{p_1} n_2 (E_2^{-1} - 1) + \\
+ \gamma_{p_1} (E_1^0 - 1) n_2 + k_{s_1} n_2 (E_2^1 - 1) + \gamma_{p_1} (E_1^0 - 1) n_4 + \\
+ \gamma_{s_2} (E_2^{-1} - 1) n_3 + k_{p_2} n_3 (E_4^{-1} - 1) + \gamma_{p_2} (E_4^1 - 1) n_4 + \\
+ k_{c_1} n_1 n_5 + \alpha \gamma_{c_1} (E_1^{-1} E_1^0 - 1) n_6 + \\
+ k_{p_2} n_5 (E_5^{-1} - 1) + (E_7^1 - 1) n_7 + \gamma_{c_2} (E_6^1 - 1) n_8 \right\} P(\{n_i\}, t) \tag{7}
\]

\[
\frac{\partial P(n_1, \tau)}{\partial \tau} = \left\{ k_s (E_1^{-1} - 1) + \gamma_s (E_1^1 - 1) n_1 + k_m (E_2^{-1} - 1) + \\
+ \gamma_m (E_1^0 - 1) n_2 + k_{c_1} (E_2^1 E_3^0 - 1) n_1 n_2 + \\
+ \alpha \gamma_{c_1} (E_2^0 - 1) n_3 + k_{p_1} n_2 (E_4^0 - 1) + \gamma_{p_1} (E_4^0 - 1) n_4 + \\
+ k_m (E_5^1 - 1) + \gamma_{c_1} (E_5^0 - 1) n_5 + \\
+ k_{c_1} (E_2^0 E_5^0 - 1) n_1 n_5 + \gamma_{c_1} (E_6^0 - 1) n_6 + \\
+ k_{p_2} n_5 (E_7^{-1} - 1) + (E_7^1 - 1) n_7 + \gamma_{c_2} (E_6^1 - 1) n_8 + \\
+ (1 - \alpha) \gamma_{c_2} (E_6^0 - 1) n_6 \right\} P(\{n_i\}, t) \tag{8}
\]

\[
\frac{\partial P(n_1, \tau)}{\partial \tau} = \left\{ k_s (E_1^{-1} - 1) + \gamma_s (E_1^1 - 1) n_1 + \\
+ k_{c_1} (E_2^0 E_3^{-1} - 1) n_1 + k_m (E_2^{-1} - 1) + \\
+ \gamma_m (E_1^0 - 1) n_2 + k_{c_1} (E_2^1 E_3^{-1} - 1) n_1 n_2 + \\
+ \alpha \gamma_{c_1} (E_2^0 - 1) n_3 + k_{p_1} n_2 (E_4^{-1} - 1) + \gamma_{p_1} (E_4^{-1} - 1) n_4 + \\
+ k_m (E_5^1 - 1) + \gamma_{c_1} (E_5^0 - 1) n_5 + \\
+ k_{c_1} (E_2^0 E_5^0 - 1) n_1 n_5 + \gamma_{c_1} (E_6^0 - 1) n_6 + \\
+ k_{p_2} n_5 (E_7^{-1} - 1) + (E_7^1 - 1) n_7 + \gamma_{c_2} (E_6^1 - 1) n_8 + \\
+ (1 - \alpha) \gamma_{c_2} (E_6^0 - 1) n_6 \right\} P(\{n_i\}, t) \tag{9}
\]

\[
\frac{\partial P(n_1, \tau)}{\partial \tau} = \left\{ k_s (E_1^{-1} - 1) + \gamma_s (E_1^1 - 1) n_1 + \\
+ k_{c_2} (E_2^0 E_3^0 - 1) n_1 + k_m (E_2^{-1} - 1) + \\
+ \gamma_m (E_1^0 - 1) n_2 + k_{c_2} (E_2^1 E_3^0 - 1) n_1 n_2 + \\
+ \alpha \gamma_{c_1} (E_2^0 E_4^0 - 1) n_3 + k_{p_1} n_2 (E_4^{-1} - 1) + \gamma_{p_1} (E_4^{-1} - 1) n_4 + \\
+ k_m (E_5^1 - 1) + \gamma_{c_1} (E_5^0 - 1) n_5 + \\
+ k_{c_2} (E_2^0 E_5^0 - 1) n_1 n_5 + \alpha \gamma_{c_2} (E_6^0 - 1) n_6 + \\
+ k_{p_2} n_5 (E_7^{-1} - 1) + (E_7^1 - 1) n_7 + \gamma_{c_2} (E_6^1 - 1) n_8 + \\
+ (1 - \alpha) \gamma_{c_2} (E_6^0 - 1) n_6 \right\} P(\{n_i\}, t) \tag{10}
\]

\[
\frac{\partial P(n_1, \tau)}{\partial \tau} = \left\{ k_s (E_1^{-1} - 1) + \gamma_s (E_1^1 - 1) n_1 + \\
+ k_{c_2} (E_2^0 E_3^0 - 1) n_1 + k_m (E_2^{-1} - 1) + \\
+ \gamma_m (E_1^0 - 1) n_2 + k_{c_2} (E_2^1 E_3^0 - 1) n_1 n_2 + \\
+ \alpha \gamma_{c_1} (E_2^0 E_4^0 - 1) n_3 + k_{p_1} n_2 (E_4^{-1} - 1) + \gamma_{p_1} (E_4^{-1} - 1) n_4 + \\
+ k_m (E_5^1 - 1) + \gamma_{c_1} (E_5^0 - 1) n_5 + \\
+ k_{c_2} (E_2^0 E_5^0 - 1) n_1 n_5 + \alpha \gamma_{c_2} (E_6^0 - 1) n_6 + \\
+ k_{p_2} n_5 (E_7^{-1} - 1) + (E_7^1 - 1) n_7 + \gamma_{c_2} (E_6^1 - 1) n_8 + \\
+ (1 - \alpha) \gamma_{c_2} (E_6^0 - 1) n_6 \right\} P(\{n_i\}, t) \tag{11}
\]

From each master equation we obtained the first two moments of the various distributions through the linear noise approximation (LNA) [32]. Briefly, given \( R \) reactions and \( N \) molecular species, a general master equation takes the form

\[
\frac{d P(\vec{n}, t)}{dt} = \Omega \sum_{i=1}^{R} \left( \prod_{j=1}^{N} \mathcal{E}_{ij}^{-v_{ij} - 1} \right) f_i(\vec{n} \Omega^{-1}, \Omega) P(\vec{n}, t) \tag{12}
\]

where \( \Omega \) is the volume of the system, \( v_{ij} \) is a matrix with dimensions \# reactions \cdot \# species (in which each element is equal to the concentration change of species \( j \) due to reaction \( i \)) and \( \mathcal{E} \) is the step-operator.
With the following change of variables
\[ \vec{n} = \Omega \vec{x} + \Omega^2 \vec{\xi} \]
and after its substitution in the master equation, we obtain
\[
\frac{\partial \Pi}{\partial t} = \Omega \sum_{i=1}^{N} \frac{dx_i}{dt} \frac{\partial \Pi}{\partial \xi_i} = \Omega \sum_{i=1}^{R} \left( \prod_{j=1}^{N} \sum_{l=0}^{\infty} \frac{(-v_{ij})^l}{l!} \Omega^{-l} \frac{\partial^l}{\partial \xi_i^l} - 1 \right) f_i(\vec{x} + \Omega^2 \vec{\xi}) \Pi(\vec{\xi}, t) .
\]

From the highest order (\(\Omega^1\)) we obtain the equations for the mean concentrations per unit of volume. From the order \(\Omega^0\) and under the assumption that \(\Omega\) is large enough (so that smaller orders are negligible) we obtain the equations for the fluctuations. Expanding the functions \(f_i\) in power of \(\xi\) we have to consider only the 0th-order:
\[
\frac{\partial \Pi}{\partial t} = -\Omega \sum_{i=1}^{R} \sum_{j=1}^{N} (\sum_{k=1}^{N} v_{ij} \frac{\partial f_i}{\partial \xi_k}) \frac{\partial}{\partial \xi_j} (\xi_k \Pi) + \sum_{j,k=1}^{N} \left( \sum_{i=1}^{R} f_i(\vec{x}) v_{ij} v_{ik} \right) \frac{\partial}{\partial \xi_j} \frac{\partial}{\partial \xi_k} \Pi
\]

Then, defining the two matrices
\[
\vec{A}_{jk} = \sum_{i=1}^{R} v_{ij} \frac{\partial f_i}{\partial \xi_k}, \quad \vec{B}_{jk} = \sum_{i=1}^{R} f_i(\vec{x}) v_{ij} v_{ik}
\]
and using the multivariate Fokker-Planck equation’s solution for the first two moments, we obtain the following relationships:
\[
\partial_t <\vec{\xi}> = \vec{A} <\vec{\xi}> \quad \text{with} \quad <\vec{\xi}> = (<\xi_1>, \ldots, <\xi_N>)^t ;
\]
\[
\partial_t \Xi = \vec{A} \Xi + \Xi \vec{A}^t + \vec{B} \quad \text{with} \quad \Xi_{ij} = <\xi_i \xi_j> .
\]

From these equations it is straightforward to calculate the approximated correlations \(r_{x,y}\) and variances \(\sigma_x\) for each concentration. In supplementary figure 1 we show the comparison between simulations and approximated analytical results.

## 3 Linear noise approximation and simulations: steady state discrepancy

The main problem of the above analysis is that the LNA does not properly take into account the correlation between chemical species in the evaluation of steady state concentrations. Indeed, if we solve the generating function for the first moments for mRNA and protein we obtain:
\[
< m_1 > = k_{m1} < s_{m1} > - k_{m1}^\text{on} < s_{m1} >
\]
\[
< p_1 > = k_{p1} < m_1 > - k_{p1}^\text{on} < s_{m1} >
\]
\[
< m_2 > = k_{m2} < s_{m2} > - k_{m2}^\text{on} < s_{m2} >
\]
\[
< p_2 > = k_{p2} < m_2 >
\]

The LNA expectation for the mixed moments is \(< xy >= < x >< y >\) while from the Master equation we find
\[
< xy >= < x >< y > + \sigma_x \sigma_y r_{xy} = (1 + \eta_x \eta_y r_{xy}) < x >< y >
\]
Figure 1. Examples of linear noise approximation and gillespie comparison, measuring correlations \( r_{m_1,m_2}, r_{s,m_1}, \) and \( r_{s,m_2} \) among \( s, m_1 \) and \( m_2 \) in the micFFL as a function of the miRNA production rate \( k_s \). Points correspond to stochastic simulations and continuous lines to LNA solutions. (a) and (b) correspond to two different sets of parameters. (a) Parameters: \( k_{m_1} = 23.5, k_{m_2} = 41, k_{on_{1}} = k_{on_{2}} = 1.5, k_{p_1} = k_{p_2} = 117, \gamma_{m_1} = \gamma_{m_2} = 2, \gamma_{s} = \gamma_{p_1} = 1, \alpha_{c_1} = \alpha_{c_2} = 1.5, (1 - \alpha)\gamma_{c_1} = (1 - \alpha)\gamma_{c_2} = 1, h = 200, n = 1 and \( k_s \) varies. (b) Parameters: \( k_{m_1} = 235, k_{m_2} = 410, k_{on_{1}} = k_{on_{2}} = 1.5, k_{p_1} = k_{p_2} = 117, \gamma_{m_1} = \gamma_{m_2} = 2, \gamma_{s} = \gamma_{p_1} = 1, \alpha_{c_1} = \alpha_{c_2} = 1.5, (1 - \alpha)\gamma_{c_1} = (1 - \alpha)\gamma_{c_2} = 1, h = 200, n = 1 and \( k_s \) is variable.

where \( \eta_s \) are measures of noise. Using equation (20) we can rewrite the steady state values corrected with a correlation term

\[
\begin{align*}
< m_1 > &= \frac{k_{m_1}}{\gamma_m + k_{on}(1+\eta_m r_{s,m_1})<s>} \\
< p_1 > &= \frac{k_{p_1}}{\gamma_{p_1}} < m_1 > \\
< m_2 > &= \frac{k_{m_2}}{\gamma_m + k_{on}(1+\eta_m r_{s,m_2})<s>} \\
< p_2 > &= k_{p_2} < m_2 >
\end{align*}
\] (21)

As it is possible to notice in Figure 2, these corrections can well explain the discrepancy occurring between simulations and approximated first order differential equations. Notice that the highest values of the correlation are obtained in the parameter region in which concentrations of microRNA and messengers are similar.

4 Comparison between NM4 and micFFL

To better investigate the increase in correlation due to the transcriptional link, we compare NM4 and micFFL (i.e. the two circuits which show the best results in terms of correlation between TF and T). We fix mRNA, protein, microRNA and complexes amounts and then evaluate the ratio between \( p_1 \) and \( p_2 \) correlations in NM4 and micFFL. The constraint is thus the following:

\[
k_{m_2}^{NM4} = k_{m_2}^{micFFL} \frac{p_2^{NM4}}{p_2^{micFFL} + h^n}.
\] (22)

Thanks to this constraint it is possible to compare directly the gain of correlation due to micFFL with respect to NM4. Our results are shown in Figure 3. The circuit with the transcriptional link between TF and T proteins always reaches higher values of correlation.
Figure 2. (a) Comparison among steady state concentrations. The concentrations of the involved species are plotted against the miRNA transcription rate $k_s$. Points are simulated data, dotted lines are values expected from the system of first order differential equations and continuous lines are values expected from the correction of the master equation. (b) Correlations between microRNA and messengers. The highest correlated region is located where concentrations of microRNA and messengers are similar. Parameters: $k_{m1} = 23.5$, $k_{m2} = 41$, $k_{ci}^{on} = k_{c2}^{on} = 1.5$, $k_{p1} = k_{p2} = 117$, $\gamma_{m1} = \gamma_{m2} = 2$, $\gamma_s = \gamma_{p1} = 1$, $\alpha\gamma_{c1} = \alpha\gamma_{c2} = 1.5$, $(1 - \alpha)\gamma_{c1} = (1 - \alpha)\gamma_{c2} = 1$, $h = 200$, $n = 1$ and $k_s$ is variable.
Figure 3. Correlation analysis of NM4 and micFFL fixing the amount of all the molecular species. Correlation values are calculated between $p_1$ and $p_2$. A Ratio between correlation of micFFL and NM4. Each curve corresponds to different values of the interaction strength F. Varying the amount of TF, micFFL keeps correlation always higher than NM4. B Correlation in micFFL varying $p_1$. C Correlation in NM4 varying $p_1$. 
5 Comparison with the explicit promoter dynamics

In all the models used we assumed that the promoter dynamics is much faster than the other reactions. In order to test if such common approximation does not affect our results we substitute the reaction for the target mRNA production with three reactions describing the promoter interaction with the transcription factor (see Table below).

| Reaction | Rate | Picture |
|----------|------|---------|
| $p_1 \to p_1 + m_2$ | $k_{m_2} \frac{p_1}{p_1+h}$ | ![Reaction Diagram](image1) |
| $p_1 + \text{Prom}_\text{off} \to \text{Prom}_\text{on}$ | $k_{prom} \frac{p_1}{p_1+h}$ | ![Reaction Diagram](image2) |
| $\text{Prom}_\text{on} \to m_2 + \text{Prom}_\text{on}$ | $k_{m_2}$ | ![Reaction Diagram](image3) |
| $\text{Prom}_\text{on} \to \text{Prom}_\text{off} + p_1$ | $k_{prom} \frac{p_1}{p_1+h}$ | ![Reaction Diagram](image4) |

From the reactions in Table 23 it is possible to calculate the probability of having the TF bound to the promoter. The activated promoter ($p_{\text{bound}}$) can then produce mRNA with a fixed rate:

$$p_{\text{bound}} = k_{\text{prom}} \frac{p_1}{p_1 + h}$$  \hspace{1cm} (24)

Comparing the two models (without and with the explicit promoter), we find the following mapping:

$$k_{m_2} \frac{p_1^n}{h^n + p_1^n} = k_{m_2} \frac{k_{\text{prom}} p_1}{k_{\text{off}} + k_{\text{prom}} p_1} \Rightarrow n = 1, h = \frac{k_{\text{prom}}}{k_{\text{off}}}. \hspace{1cm} (25)$$

Figure 4 shows that for fast promoter dynamics there is a very good agreement between the two models in terms of mean number of molecules (Figure 4A and B), noise (Figure 4C) and correlation coefficient (Figure 4D). Each curve is the mean over 1000 Gillespie’s trajectories.

6 Stability analysis

In this section we accomplish a brief stability analysis of the micFFL circuit. From the definition

$$\frac{d}{dt} \vec{x} = \vec{F}(\vec{x})$$  \hspace{1cm} (26)

we expand the vectors for the micFFL case, so that

$$\begin{align*}
\frac{ds}{dt} &= k_s - \gamma_s s - k_{c_1} s m_1 + \alpha \gamma_{c_1} c_1 - k_{c_2} s m_2 + \alpha \gamma_{c_2} c_2 \\
\frac{dm_1}{dt} &= k_{m_1} - \gamma_m m_1 - k_{c_1} s m_1 \\
\frac{dc_1}{dt} &= k_{c_1} s m_1 - \gamma_{c_1} c_1 \\
\frac{dp_1}{dt} &= k_{p_1} m_1 - \gamma_{p_1} p_1 \\
\frac{d_{m_2}}{dt} &= k_{m_2} p_1 + p_2 - \gamma_{m_2} m_2 - k_{c_2} s m_2 \\
\frac{dc_2}{dt} &= k_{c_2} s m_2 - \gamma_{c_2} c_2 \\
\frac{dp_2}{dt} &= k_{p_2} m_2 - p_2
\end{align*}$$  \hspace{1cm} (27)
Figure 4. Comparison between Gillespie simulations with promoter model or with Michelis-Menten function. Parameters: $k_m = 23.5, k_{m2} = 41, k_{c1}^{on} = k_{c2}^{on} = 1.5, k_{p1} = k_{p2} = 117, \gamma_m = \gamma_{m2} = 2, \gamma_s = \gamma_{p1} = 1, \alpha \gamma_{c1} = \alpha \gamma_{c2} = 1.5, (1-\alpha)\gamma_{c1} = (1-\alpha)\gamma_{c2} = 1, k_{on} = 6, k_{prom} = 1000, h = 200, n = 1$ and $k_s$ is variable.
Depending on the sign of $\nabla \cdot \vec{F}$ the system could be dissipative ($\nabla \cdot \vec{F} \leq 0$) or conservative ($\leftrightarrow \nabla \cdot \vec{F} = 0$).

In our particular case the system is dissipative:

$$\nabla \cdot \vec{F}(x) = -\gamma_{c_1} - \gamma_{c_2} - \gamma_s - \gamma_{m_1} - \gamma_{m_2} - \gamma_{p_1} - (k_{c_1}^{\text{on}} + k_{c_2}^{\text{on}})s - k_{c_1}^{\text{on}}m_1 - k_{c_2}^{\text{on}}m_2 < 0.$$  \hspace{1cm} (28)

Since the divergence of $\vec{F}$ is always negative (the parameters have to be always positive to make sense), the phase-space is not conserved and a 7-dimensional attractor exists. Further information on the stability of the system can be obtained if one fixes the values of the parameters in the equations. For example, with the set of parameters we used in our numerical experiments ($k_{m_1} = 23.5, k_{m_2} = 41, k_s = 17.5, k_{c_1}^{\text{on}} = k_{c_2}^{\text{on}} = 1.5, k_{p_1} = k_{p_2} = 117, \gamma_{m_1} = \gamma_{m_2} = 2, \gamma_s = \gamma_{p_1} = 1, \alpha \gamma_{c_1} = \alpha \gamma_{c_2} = 1.5, (1 - \alpha)\gamma_{c_1} = (1 - \alpha)\gamma_{c_2} = 1, h = 200, n = 1$), the system has a linearly stable fixed point.

7 Steady state analysis with the logic approximation

We discuss here the steady state analysis of the Eq.(1) of the main text in the framework of the logic approximation in which the Hill function is approximated with the Heaviside step function $f(p_1) = H(p_1 - h)$. Even if this approximation is very crude it may help to have an intuitive picture of the behaviour of the various players of the circuit as a function of the parameters of the circuit. The equations for $p_1$ and $p_2$ (see Eq.(1) of the main text) can be solved immediately leading to the steady state values: $p_1^0 = k_p m_1 / \gamma_{p_1}$ and $p_2^0 = k_p m_2 / \gamma_{p_2}$. We can rescale the activation coefficient $h_s \equiv h_{p_1} / k_{p_1}$ and then write the step function as a function of $m_1$ and eliminate $p_1$ from the equations. Following [16] we introduce the quantities ($i = 1, 2$)

$$\lambda_i \equiv \frac{k_{c_i}^{\text{on}}}{\gamma_{c_i}},$$

$$\theta_i \equiv \frac{\gamma_{c_i}}{\gamma_{m_i}} M_{\text{tot}},$$

which have an immediate physical interpretation: $\theta_i$ is the (suitably rescaled) amount of miRNA acting on $m_i$ and $1/\lambda_i$ measures the strenght of this interaction, i.e. the lifetime of the complex $c_i$. These will be in the following the only external parameters of micFFL. Finally we assume for simplicity $\lambda_1 = \lambda_2 = \lambda$, $\theta_1 = \theta_2 = \theta$, and denote $m_1^0$ as the steady state value $m_i$ would reach if $M_{\text{tot}} = 0$ (i.e. $m_1^0 \equiv k_{m_1} / \gamma_{m_1}$ and $m_2^0 \equiv k_{m_2} / \gamma_{m_2}$ if $m_1^0 > h_s$ and $m_2^0 = 0$ otherwise). Then it is easy to obtain the steady state values of $m_1$ e $m_2$ as a function of $\theta$ and $\lambda$.

$$m_1 = m_1^0 + \frac{m_2^0 - \theta - \lambda + \sqrt{(m_1^0 + m_2^0 - \theta - \lambda)^2 + 4(m_1^0 + m_2^0)\lambda}}{2(m_1^0 + m_2^0)}$$

$$m_2 = m_2^0 + \frac{m_1^0 - \theta - \lambda + \sqrt{(m_1^0 + m_2^0 - \theta - \lambda)^2 + 4(m_1^0 + m_2^0)\lambda}}{2(m_1^0 + m_2^0)}$$

The implications of this result can be better appreciated if we take the $\lambda \to 0$ limit.

$$m_1 = \begin{cases} 0 & m_1^0 + m_2^0 \leq \theta \\ m_1^0 (1 - \theta / m_1^0 + m_2^0) & m_1^0 + m_2^0 > \theta \end{cases}$$

$$m_2 = \begin{cases} 0 & m_1^0 + m_2^0 \leq \theta \land m_1 < h_s \\ m_2^0 (1 - \theta / m_1^0 + m_2^0) & m_1^0 + m_2^0 > \theta \land m_1 > h_s \end{cases}$$

with $m_0^0 = \begin{cases} 0 & m_1 < h_s \\ m_2^0 & m_1 > h_s \end{cases}$

We plot the value of $m_1$ and $m_2$ as a function of $\theta$ (i.e. of the miRNA concentration) in fig.s 4a and 4b. Looking at these figures we see a few interesting and non trivial features:
Figure 5. Steady state analysis with the logic approximation of the micFFL. Plots A and B show the mRNA concentrations, respectively, of transcription factor ($m_1$) and target ($m_2$) as a function of the microRNA concentration ($\theta$) in the limit $\lambda \to 0$. $H_s$ represents the activation threshold of the Heaviside function.

- In the $\lambda \to 0$ limit we find for the transcription factor $m_1$ the same threshold behaviour discussed in [16] as a function of the miRNA concentration. The same effect should be present also in the target concentration $m_2$, but is hidden by the fictitious step behaviour due to the logic approximation. It is easy to understand the origin of this threshold behaviour: if the number of free miRNA molecules greatly exceeds the number of transcripts $m_{TF}$ and $m_{T}$, then these will be almost all bound in complexes and the corresponding proteins will not be expressed. On the opposite side, if the number of $m_{TF}$ and $m_{T}$ molecules overcomes miRNA amount, then nearly all miRNAs will be bound in complexes but there will be a sufficient amount of free $m_{TF}$s and $m_{T}$s to be translated.

- As the total miRNA concentration decreases the TF concentration increases following the trajectory plotted in fig 5. When the TF concentration reaches the threshold $h_s$ for the $m_2$ activation we observe a sudden enhancement in the TF concentration due to the sponge interaction between $m_1$ and $m_2$. When also $m_2$ is present then the two mRNAs start to compete for the same miRNAs and as a net effect there is a smaller amount of miRNA available to downregulate $m_1$. This non linear behavior of the TF concentration as the mRNA concentration increases is in our opinion one of the most effective ways to detect sponge-like interactions.

- The ratio $m_2/m_1$ (and thus $p_2/p_1$) can only take two possible values: $m_2/m_1 = m_2^0/m_1^0$ for $m_1 > h_s$ and $m_2/m_1 = 0$ for $m_1 < h_s$. However this is clearly an artifact of the logic approximation.