Regulation by small RNAs via coupled degradation: mean-field and variational approaches

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Regulatory genes called small RNAs (sRNAs) are known to play critical roles in cellular responses to changing environments. For several sRNAs, regulation is effected by coupled stoichiometric degradation with messenger RNAs (mRNAs). The nonlinearity inherent in this regulatory scheme indicates that exact analytical solutions for the corresponding stochastic models are intractable. Here, we present a variational approach to analyze a well-studied stochastic model for regulation by sRNAs via coupled degradation. The proposed approach is efficient and provides accurate estimates of mean mRNA levels as well as higher order terms. Results from the variational ansatz are in excellent agreement with data from stochastic simulations for a wide range of parameters, including regions of parameter space where mean-field approaches break down. The proposed approach can be applied to quantitatively model stochastic gene expression in complex regulatory networks.

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A new paradigm for cellular regulation has emerged in recent years with the discovery of novel non-coding genes called small RNAs (sRNAs). In bacteria, sRNAs often function as global regulators that mediate cellular adaptation to changing environments [1]. In higher organisms, the corresponding genes (microRNAs) are known to play key roles in the regulation of critical processes such as development, stem cell pluripotency and cancer [2,3]. It has been proposed that one of the key functions of sRNAs in controlling cellular processes is to regulate the variability (noise) in gene expression [3]. Recent experimental developments have led to approaches for quantifying such variability using single-molecule measurements of mRNA levels [4]. These technological advances have now made possible experimental studies that analyze the roles of sRNAs in noise regulation during important processes such as development. Correspondingly, there is a need for theoretical approaches that complement such experimental efforts to enable a quantitative understanding of different mechanisms of sRNA-based regulation.

While the molecular mechanisms of sRNA-mediated regulation continue to be investigated, one established mechanism, representative of several bacterial sRNAs, corresponds to binding with mRNAs followed by coupled stoichiometric degradation [5]. An important challenge for current research is to analyze how this regulatory mechanism impacts the variability of gene expression across a population of cells. Several recent theoretical studies [6–11] have analyzed models based on the corresponding reaction scheme (shown in Fig. 1A). The nonlinearity inherent in this reaction scheme implies that exact analytical solutions for the corresponding stochastic model are intractable; thus approximate analytical approaches are needed. Previous theoretical studies have primarily focused on mean-field (MF) approaches and on steady-state distributions using expansions around MF solutions. However, MF approaches are not accurate when we have a combination of nonlinear reaction rates (due to interaction with small RNAs) and low mRNA/sRNA levels, which points to the need for development of alternative analytical approaches.

In this paper, we analyze stochastic models of sRNA-based regulation via coupled degradation (as shown in Fig. 1A). We first discuss the MF approximation, which corresponds to neglecting mRNA-sRNA correlations, and define dimensionless variables that are useful in quantifying deviations between MF results and data from stochastic simulations. To go beyond MF, we use a variational approach which has been successfully applied to gene regulatory networks in recent work [12–15]. Within this approach, we present a general ansatz for the steady-state probability distribution which, at the simplest level, reduces to the MF approximation. At the next level, the variational ansatz gives results that are in excellent agreement with data from simulations for the mean and variance of the regulated mRNA distribution. The proposed method can be used for efficient and accurate quantitative analysis of sRNA-based regulation of gene expression.

We begin by considering the kinetic scheme presented in Fig. 1A. The probability distribution of mRNA and
sRNA levels per cell, \( P_{m,s}(t) \), obeys the master equation:

\[
\partial_t P_{m,s} = k_m P_{m-1,s} + k_s P_{m,s-1} + \mu_m (m + 1)P_{m+1,s} + \mu_s (s + 1)P_{m,s+1} + \gamma (m + 1)(s + 1)P_{m+1,s+1} - (k_m + k_s + \mu_m m + \mu_s s + \gamma m s)P_{m,s},
\]

where \( k_j, \mu_j (j = m, s) \) and \( \gamma \) are the parameters defined in Fig. 1A. We will focus on the stationary distribution, denoted by \( P^*_{m,s} \). It is convenient to define the following set of independent dimensionless parameters: \( \epsilon_m = k_s \gamma / \mu_m \mu_s \), \( \epsilon_s = k_m \gamma / \mu_m \mu_s \) and \( n_j = k_j / \mu_j \) \( (j = m, s) \). From the master equation (1), we can explicitly relate the average mRNA and sRNA levels to the correlation term \( \langle ms \rangle \) [16, 17] via:

\[
\frac{1}{\epsilon_m} \left( 1 - \frac{\langle m \rangle}{n_m} \right) = \frac{1}{\epsilon_s} \left( 1 - \frac{\langle s \rangle}{n_s} \right) = \frac{\langle ms \rangle}{n_m n_s}, \tag{2}
\]

where \( \langle \cdot \rangle \) denotes the stationary average. More generally, moments at one level are coupled to higher-order moments due to the nonlinear interaction term. This hierarchy makes the exact solution of the master equation intractable. Defining \( X = \langle m \rangle / n_m \), \( Y = \langle s \rangle / n_s \), and \( C = \langle ms \rangle / \langle m \rangle \langle s \rangle \), equation (2) leads to

\[
1 - \frac{X}{\epsilon_m} = 1 - \frac{Y}{\epsilon_s} = C XY. \tag{3}
\]

Traditionally, a first approximation, known as the MF approximation, consists of neglecting correlations through the substitution \( \langle ms \rangle \rightarrow \langle m \rangle \langle s \rangle \). The MF assumption thus corresponds to \( C = 1 \) and leads to

\[
\epsilon_m XY + X - 1 = 0, \quad \epsilon_s XY + Y - 1 = 0. \tag{4}
\]

Comparing Eqns. (3) and (4), we see that the exact means (i.e. solutions of Eqn. (3)) are generated by the MF solutions considered with the rescaled interaction parameter \( \gamma' = C \gamma \). Determination of \( C \) can therefore provide accurate estimates of the mean mRNA and sRNA levels. The ratio \( C \) is also an indicator of the accuracy of MF: it is a good approximation when \( C \approx 1 \), whereas deviations from unity indicate that better approximations are needed.

Furthermore, note that \( X \) and \( Y \) are, in general, functions of the four parameters \( \epsilon_m, \epsilon_s, n_m \) and \( n_s \); however the MF approximation (Eq. 4) predicts that both quantities depend only on \( \epsilon_m \) and \( \epsilon_s \). It follows that MF theory breaks down in regions of parameter space where \( X \) and \( Y \) depend on the parameters \( n_m \) and \( n_s \) (for fixed \( \epsilon_m \) and \( \epsilon_s \)). These regions are indicated by significant deviations between the exact ratio \( X / Y \) and the solution \( \lambda_+ / (\lambda_-) \) of Eq. (4).

We now analyze deviations of the MF results from stochastic simulations data obtained using the Gillespie algorithm [18]. The ratios \( X \) and \( C \) are plotted in figures 1B and 2A respectively. These data are presented as a function of \( n_m \) and \( n_s \), keeping \( \epsilon_m \) and \( \epsilon_s \) constant. The figures indicate that both quantities converge towards the MF predictions in the limit \( n_s, n_m \rightarrow 0 \) \((X \rightarrow 0.618 \text{ and } C \rightarrow 1)\). More significantly, the data shows that MF is not a good approximation for small \( n_m \) and \( n_s \). This is important to note since, in several cellular systems, mRNA abundances can be low (i.e. \( n_m \) is small) [19]. This indicates that more accurate approximation are needed in such cases.

Furthermore, in the uncorrelated approximation, the stationary probability distribution can be written as the product of Poisson distributions \( \Pi_{\lambda_+}(m) \times \Pi_{\lambda_-}(s) \), where \( \Pi_{\lambda}(n) = e^{-\lambda} n^n / n! \). Defining the marginal distributions \( P^*_{m,s} = \sum_s P^*_m \) and \( P^*_m = \sum_m P^*_m \), the ratio \( d_j = \langle j \rangle / (\langle j^2 \rangle - \langle j \rangle^2) \) \( (j = m, s) \) measures deviations between the marginals \( P^*_m \) and the simple Poisson distribution. Again, deviations of \( D = d_s \times d_m \) from unity reveal that both marginal probability distributions cannot be approximated by the Poisson distribution. In Fig. 2B, stochastic simulations data indicate that the coefficient \( D \) deviates significantly from one for large \( n_m \) and \( n_s \). This observation implies that higher-order terms, such as \( \langle m^2 \rangle \) and \( \langle s^2 \rangle \) cannot be obtained using the MF prediction \( (j^2) - (j) = (j) \) \( (j = m, s) \), even in regions of parameter space for which the mean values are given accurately by the MF approximation. Interestingly, it is for small parameter values \( n_j \) \( (j = m, s) \), for which the MF approximation does not give accurate mean values, that \( D \) converges to one. This observation is an indication that the Poisson distribution is in some way embedded in the structure of \( P^*_{m,s} \).

Based on the preceding analysis, it seems natural to approximate \( P^*_{m,s} \) as a superposition of Poisson distributions. This approximation can be implemented using the variational method introduced by Eyink [20], combined with the quantum Hamiltonian formalism of the master
FIG. 2: Stationary value of $C = \langle ms \rangle / \langle m \rangle \langle s \rangle$ (A) and $D = d_m \times d_s$ (B), obtained from simulation data, plotted as a function of $n_m$ and $n_s$. We keep $\epsilon_m = \epsilon_s = 1$ and $\gamma = 1$.

equation 12 13. Following the mapping outlined by Doi [21], we define the operators $a^\dagger$ and $a$ (respectively $b^\dagger$ and $b$) associated with the creation and annihilation of mRNA (sRNA). The master equation 11 takes the compact form $\dot{\psi}(t) = -\mathcal{L}\psi(t)$ with

$$\mathcal{L} = \sum_{i,j=0}^d \Theta_{i,j} e^{\alpha_i(a^{i\dagger} - 1)} e^{\beta_j(b^{j\dagger} - 1)} |0,0\rangle,$$

with $\Lambda_R = \{\alpha_p, \beta_q, \Theta_{p.q}\}$ and $\Lambda_L = \{\theta_{p.q}\}$ ($\theta_{d,d} = 0$). In each vector, the total number of parameters $N$ is given by $N = d(d + 2)$. The parameters of $\phi_L$ are imposed by the condition $\langle \phi_L | m, n \rangle = 1$ which leads to $\theta_{p,q} = 0, \forall p, q$. It follows that the set $\Lambda_R$ is solution of $\langle \delta \phi_L | \mathcal{L} | \phi_R \rangle |_{\Lambda_L = \{0\}} = 0$. Our calculation leads to the system of equations:

$$\sum_{p,q=1}^d \Theta_{p,q} \alpha_p \beta_q \times [\epsilon_s \epsilon_m (ij + i\beta_q + j\alpha_p) + n \epsilon_m (1 - n/\beta_q)] = 0,$$

generated for $i, j = 0, 1, 2, ..., d$ with the pair $(i = d, j = d)$ excluded. The first equation (for $i = j = 0$) corresponds to the probabilistic interpretation: $\langle \phi_L | \phi_R \rangle = 1$ and leads to the normalization constraint $\sum_{p,q} \Theta_{p,q} = 1$. From equation 8, one can then generate the $N$ independent conditions required to determine the right eigenvector parameters. It follows that an approximation of the stationary distribution is given by $\mathcal{P}_{m,s} = \langle m, s | \mathcal{L} | \phi_R \rangle / \langle m | \phi_R \rangle$, where $\Lambda_R = \{\alpha_p, \beta_q, \Theta_{p,q}\}$ is solution of (8). The latter distribution can be explicitly written as a superposition of Poisson distributions:

$$\mathcal{P}_{m,s} = \sum_{p,q} \Theta_{p,q} \Pi_{m,s}(m)|\phi_R \rangle.$$
To compare our results in the non-symmetric case, we consider variations in $\mu_m$, keeping $\mu_s = 2$ and $\gamma = 1$ fixed. The set of parameters is once again computed numerically, solving 8 coupled equations generated from equation (9). The ratio $\epsilon_s$ is kept equal to unity while $\epsilon_m = 4$, 1 and 1/4. As shown in Fig. 3B, the ansatz predictions are, once again, in excellent agreement with simulation data.

In conclusion, we have presented a variational approach for analyzing a coupled degradation mechanism of sRNA-based regulation. The latter method generates a set of algebraic equations that can be solved numerically. At the simplest level, the approach reduced to the MF approximation which is shown to be inaccurate for low abundances of the interacting components. The approach proposed allows for systematic improvements over MF and, at the next level, gives excellent agreement with simulation data for the mean and variance of steady-state mRNA/sRNA distributions. The results derived will aid approaches for inference of model parameters from experimental measurements of mean and variance. More generally, the proposed approach can be extended to treat other biological networks with nonlinear interactions for which analytical solutions of the corresponding stochastic models are intractable. In such cases, the proposed procedure of constructing the variational ansatz (i.e. superposition of MF probability distributions) can lead to accurate estimates of the mean and variance for quantities of interest. It is hoped that future work coupling such approaches with experiments will lead to quantitative understanding of gene expression in complex networks.

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**FIG. 3:** Comparisons of simulation data (symbols), ansatz predictions (lines) and MF results (dashed line). (A) The quantities $X = \langle m \rangle/n_m$, $C = \langle ms \rangle/\langle m \rangle s$ and $D = d_s \times d_m$ are plotted as a function of $\mu = \mu_m = \mu_s$ on a logarithmic scale, for $\gamma = 1$ (circles), $\gamma = 5$ (squares), and $\gamma = 10$ (diamonds). We keep $\epsilon_m = \epsilon_s = 1$ with $k_m = k_s = k$. (B) The quantities $X$ (left) and $C$ (right) are plotted as a function of $\mu_m$ on a logarithmic scale, for $\epsilon_m = 4$ (top), $\epsilon_m = 1$ (middle) and $\epsilon_m = 1/4$ (bottom). We keep $\mu_s = 2$, $\gamma = 1$ and $\epsilon_s = 1$.