# Deterministic model derivation and model reduction of an activator-repressor genetic oscillator

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## Introduction

This note contains the derivation of the deterministic model and model reduction using singular perturbation used in the submission "Loading as a design parameter for genetic circuits" to the 2016 American Control Conference by the same authors.

## Derivation of model

A deterministic ODE model of an activator-repressor (A-R) genetic oscillator is derived considering the biochemical reactions of activation, repression, multimerization, transcription, and translation of a generic protein (P) which, due to the symmetry of the model (both proteins are activated by A and repressed by R) can be used to describe the evolution of the concentration of both A and

R. These reactions are given by:

$$\mathrm{A} + \mathrm{A} + ... + \mathrm{A} \underset{\beta_{\mathrm{A}'}}{\overset{\beta_{\mathrm{A}}}{\rightleftharpoons}} \mathrm{A}_n, \tag{1}$$

$$\mathrm{R} + \mathrm{R} + ... + \mathrm{R} \underset{\beta_{\mathrm{R}'}}{\overset{\beta_{\mathrm{R}}}{\rightleftharpoons}} \mathrm{R}_m, \tag{2}$$

$$\mathrm{R}_m + \mathrm{DNA}^{\mathrm{P}} \underset{\mathrm{d}^*}{\overset{\mathrm{a}^*}{\rightleftharpoons}} \mathrm{R}_m : \mathrm{DNA}^{\mathrm{P}}, \tag{3}$$

$$\mathrm{A}_n + \mathrm{DNA}^{\mathrm{P}} \underset{\mathrm{d}'}{\overset{\mathrm{a}'}{\rightleftharpoons}} \mathrm{A}_n : \mathrm{DNA}^{\mathrm{P}}, \tag{4}$$

$$\mathrm{A}_n : \mathrm{DNA}^{\mathrm{P}} \xrightarrow{\alpha_1} \mathrm{m_P} + \mathrm{A}_n : \mathrm{DNA}^{\mathrm{P}}, \tag{5}$$

$$\mathrm{DNA}^{\mathrm{P}} \xrightarrow{\alpha_2} \mathrm{m_P} + \mathrm{DNA}^{\mathrm{P}}, \tag{6}$$

$$\mathrm{m_P} \xrightarrow{\kappa} \mathrm{m_P} + \mathrm{P}, \tag{7}$$

$$\mathrm{m_P} \xrightarrow{\delta} \emptyset, \tag{8}$$

$$\mathrm{P} \xrightarrow{\gamma} \emptyset. \tag{9}$$

Let A and R multimerize with cooperativity n and m, with forward rates of $\beta_A, \beta_R$ and reverse rates of $\beta'_A, \beta'_R$, respectively, leading to reactions (1)-(2). Since activation and repression are assumed to take place at the transcriptional level, the complex formed by the reversible reaction (with forward rate $a^*$ and reverse rate $d^*$) between $\mathrm{R_m}$ and DNA promoter ($\mathrm{DNA^P}$), denoted $\mathrm{R_m}$:$\mathrm{DNA^P}$, does not contribute to transcription and effectively sequesters free $\mathrm{DNA^P}$, as given in (3). Conversely, $\mathrm{A_n}$:$\mathrm{DNA^P}$ is the complex formed by the reversible reaction (with forward rate $a'$ and reverse rate $d'$) between $\mathrm{A_n}$ and $\mathrm{DNA^P}$, as shown in (4). This complex undergoes translation at rate $\alpha_1$ to produce an mRNA molecule, leading to (5). The model also assumes that some transcription can occur without A bound to $\mathrm{DNA^P}$ (i.e., transcriptional leakiness), described by (6). Translation occurs at a rate $\kappa$, given in (7), and mRNA and protein decay at a rate $\delta$ and $\gamma$, respectively, given in (8)-(9). The ODE model for the mRNA and protein dynamics is given by:

$$\begin{aligned} \dot{m}_P &= \alpha_1[A_n : DNA^P] + \alpha_2[DNA^P] - \delta m_P, \\ \dot{P} &= \kappa m_P - \gamma P. \end{aligned} \tag{10}$$

Assuming the total concentration of DNA is constant, the following conservation law holds:

$$DNA_{tot} = DNA^P + [R_m : DNA^P] + [A_n : DNA^P].$$

Assuming complex formation occurs significantly faster than mRNA and protein dynamics [1], setting their respective rate equations at quasi-steady state (i.e., $\dot{A}_n, \dot{R}_m, [\mathrm{A_n} : \dot{\mathrm{DNA}}^{\mathrm{P}}], [\mathrm{R_m} : \dot{\mathrm{DNA}}^{\mathrm{P}}] = 0$) and solving for [$\mathrm{A_n}$:$\mathrm{DNA^P}$] and [$\mathrm{DNA^P}$]

in terms of $A, R$ yields:

$$[\mathrm{A}_n : \mathrm{DNA}^{\mathrm{P}}] = \frac{\frac{a'\beta_A}{d'\beta_{A'}} DNA_{tot} A^n}{1 + \frac{a'\beta_A}{d'\beta_{A'}} A^n + \frac{a^*\beta_R}{d^*\beta_{R'}} R^m}, \tag{11}$$

$$[\mathrm{DNA}^{\mathrm{P}}] = \frac{DNA_{tot}}{1 + \frac{a'\beta_A}{d'\beta_{A'}} A^n + \frac{a^*\beta_R}{d^*\beta_{R'}} R^m}. \tag{12}$$

Equation (10) represents the dynamics of a general mRNA and protein system with transcriptional activation and repression by A and R, respectively. Substituting (11)-(12) in (10) and then using the subscripts "R" or "A" to denote parameters corresponding to R or A production and decay, respectively yields the final model equations:

$$\begin{aligned}
\dot{m}_A &= \frac{\alpha(A/k_A)^n + \alpha_0}{1 + (A/k_A)^n + (R/k_R)^m} - \delta_A m_A, \\
\dot{m}_R &= \frac{\alpha(A/k_A)^n + \alpha_0}{1 + (A/k_A)^n + (R/k_R)^m} - \delta_R m_R, \\
\dot{A} &= \kappa_A m_A - \gamma_A A, \\
\dot{R} &= \kappa_R m_R - \gamma_R R.
\end{aligned} \tag{13}$$

# Model reduction via singular perturbation of system with load to A

We consider A transcriptionally regulating downstream promoter sites. Let the free promoter sites be denoted as $C_{10}$ and the sites bound to A be denoted as $C_{11}$. Since DNA does not decay, the total concentration of promoter sites is conserved, that is $C_{10} + C_{11} = C_{t1}$, where $C_{t1}$ represents the total concentration of the free and bound promoter sites. The complex formation reaction is given by: $C_{10} + A \underset{d}{\overset{a}{\rightleftarrows}} C_{11}$, leading to the three-state system:

$$\begin{aligned}
\dot{A} &= \frac{\kappa_A}{\delta_A} \frac{\alpha(A/k_A)^n + \alpha_0}{1 + (A/k_A)^n + (R/k_R)^m} - \gamma_A A - \dot{C}_{11}, \\
\dot{R} &= \frac{\kappa_R}{\delta_R} \frac{\alpha(A/k_A)^n + \alpha_0}{1 + (A/k_A)^n + (R/k_R)^m} - \gamma_R R, \\
\dot{C}_{11} &= a(C_{t1} - C_{11})A - dC_{11}.
\end{aligned} \tag{14}$$

In order to analyze how the eigenvalues of the linearized system change due to the addition of $C_{t1}$, we consider a reduced order model. Using the assumption that complex formation ($\mathrm{C}_{11}$) occurs relatively faster than protein dynamics (A,R) [1], the three-state system can be reduced to two states. To this end, we employ singular perturbation and introduce the new (slow) variable $Z$, defined as $Z = A + C_{11}$. Rewrite the system by defining $\epsilon = \frac{\gamma_A}{d}$, $K_{d1} = \frac{d}{a}$, and $a = \frac{\gamma_A}{\epsilon K_{d1}}$.

Substituting these expressions into (14) yields the system in standard singular perturbation form given by:

$$\begin{aligned}
\dot{Z} &= \frac{\kappa_A}{\delta_A}\frac{\alpha(\frac{Z-C_{11}}{k_A})^n+\alpha_0}{1+(\frac{Z-C_{11}}{k_A})^n+(R/k_R)^m}-\gamma_A(Z-C_{11}),\\
\dot{R} &= \frac{\kappa_R}{\delta_R}\frac{\alpha(\frac{Z-C_{11}}{k_A})^n+\alpha_0}{1+(\frac{Z-C_{11}}{k_A})^n+(R/k_R)^m}-\gamma_R R,\\
\epsilon\dot{C}_{11} &= \frac{\gamma_A}{K_{d1}}(C_{t1}-C_{11})(Z-C_{11})-\gamma_A C_{11}. \qquad (15)
\end{aligned}$$

Setting $\epsilon = 0$ and solving for $C_{11}$ in terms of A yields the slow manifold:

$$C_{11} = \frac{C_{t1}A/K_{d1}}{1+A/K_{d1}} = g_1(A),$$

which can be shown to be locally exponentially stable [2]. Since $Z = A + C_{11}$, we have $\dot{Z} = \dot{A} + \dot{C}_{11}$, and so:

$$\dot{Z} = \dot{A} + \frac{dg_1(A)}{dA}\dot{A}.$$

Solving for $\dot{A}$ yields:

$$\begin{aligned}
\dot{A} &= \frac{\dot{Z}}{1+\frac{dg_1(A)}{dA}},\\
&= \Big(\frac{\kappa_A}{\delta_A}\frac{\alpha(\frac{A}{k_A})^n+\alpha_0}{1+(\frac{A}{k_A})^n+(\frac{R}{k_R})^m}-\gamma_A A\Big)\frac{(1+\frac{A}{K_{d1}})^2}{(1+\frac{A}{K_{d1}})^2+\frac{C_{t1}}{K_{d1}}}.
\end{aligned}$$

The resulting reduced model of the clock with load on A is thus given by:

$$\begin{aligned}
\dot{A} &= \frac{(1+\frac{A}{K_{d1}})^2}{(1+\frac{A}{K_{d1}})^2+\frac{C_{t1}}{K_{d1}}}\left(\frac{\kappa_A}{\delta_A}\frac{\alpha(\frac{A}{k_A})^n+\alpha_0}{1+(\frac{A}{k_A})^n+(\frac{R}{k_R})^m}-\gamma_A A\right),\\
\dot{R} &= \frac{\kappa_R}{\delta_R}\frac{\alpha(A/k_A)^n+\alpha_0}{1+(A/k_A)^n+(R/k_R)^m}-\gamma_R R.
\end{aligned}$$

Model reduction of the system with load on R can be similarly derived.

# Model reduction via singular perturbation of system with load and complex decay

We consider load to A transcriptionally regulating downstream promoter sites that decay at a constant rate when bound with A. The modified load reactions to A are given by:

$$\begin{aligned}
A + C_{10} &\underset{d}{\overset{a}{\rightleftarrows}} C_{11},\\
C_{11} &\xrightarrow{\pi_A} C_{10}.
\end{aligned}$$

The dynamics of the three-state system have changed to ($f_1(A,R) = \frac{\kappa_A}{\delta_A}\frac{\alpha(A/k_A)^n+\alpha_0}{1+(A/k_A)^n+(R/k_R)^m}$, $f_2(A,R) = \frac{\kappa_R}{\delta_R}\frac{\alpha(A/k_A)^n+\alpha_0}{1+(A/k_A)^n+(R/k_R)^m}$):

$$\begin{aligned}
\dot{A} &= f_1(A,R) - \gamma_A A + dC_{11} - aA(C_{t1} - C_{11}),\\
\dot{R} &= f_2(A,R) - \gamma_R R,\\
\dot{C}_{11} &= aA(C_{t1} - C_{11}) - (d+\pi_A)C_{11}.
\end{aligned}$$

Introduce a slow (X) and fast (Y) variable, given by:

$$\begin{aligned}
X &= A + C_{11},\\
Y &= \frac{d}{d+\pi_A}C_{11}.
\end{aligned}$$

The three-state system is thus now given by ($p_A = \frac{d}{d+\pi_A}$):

$$\begin{aligned}
\dot{X} &= f_1(X - \frac{Y}{p_A}, R) - \gamma_A(X - \frac{Y}{p_A}) - \frac{\pi_A Y}{p_A},\\
\dot{Y} &= p_A a(X - \frac{Y}{p_A})(C_{t1} - \frac{Y}{p_A}) - d\frac{Y}{p_A},\\
\dot{R} &= f_2(X - \frac{Y}{p_A}, R) - \gamma_R R.
\end{aligned}$$

Define $\epsilon = \frac{\gamma_A}{d}$, $K_{d1} = \frac{d}{a}$. This leads to $d = \frac{\gamma_A}{\epsilon}$, $a = \frac{\gamma_A}{\epsilon K_{d1}}$, and:

$$\epsilon\dot{Y} = \frac{\gamma_A p_A}{K_{d1}}A(C_{t1} - \frac{Y}{p_A}) - \frac{\gamma_A Y}{K_{d1}}.$$

Set $\epsilon = 0$ to find the slow manifold:

$$\begin{aligned}
Y &= \frac{p_A^2}{K_{d1}}A(C_{t1} - \frac{Y}{p_A}),\\
&= \frac{p_A^2 C_{t1} A}{K_{d1} + p_A A} = h_1(A).
\end{aligned}$$

Solving for $\dot{A}$:

$$\begin{aligned}
X &= A + \frac{Y}{p_A},\\
\dot{X} &= \dot{A} + \frac{1}{p_A}\frac{\partial h_1}{\partial A}\dot{A} \implies \dot{A} = \frac{\dot{X}}{1 + \frac{1}{p_A}\frac{\partial h_1}{\partial A}},\\
\dot{A} &= \frac{f_1(A,R) - \gamma_A A - \frac{\pi_A p_A C_{t1} A}{K_{d1}+p_A A}}{1 + \frac{p_A K_{d1} C_{t1}}{(K_{d1}+p_A A)^2}}.
\end{aligned}$$

Model reduction of the system with load on R and complex decay can be similarly derived.

# References

[1] U. Alon, "An Introduction to Systems Biology: Design Principles of Biological Circuits," *Chapman & Hall/CRC*, 2007.

[2] D. del Vecchio and R. Murray, "Biomolecular Feedback Systems," *Princeton University Press*, 2014.