SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY

BIO304: SYSTEMS BIOLOGY

Project 2

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Abstract

The Tet-On Doxycyclin-inducible gene expression system is one of the most powerful, versatile, and widely cited inducible mammalian expression system. It is based on a method of inducible gene expression where transcription is reversibly turned on in the presence of the antibiotic doxycycline or its derivatives. In this paper, we model the doxycyclin -induced expression systems with or without feedback and simulate their heterogeneity.

In part 1, we establish the model of doxycyclin-induced GFP expression system without feedback. We use 6 reactions to construct the model with time delay and make necessary simplifications. Then we propose one ODE to characterize these reactions. The stimulation result suggests that in such inducible system without feedback, the doxycycline efffective range is rather narrow, and the induction response is ultrasensitive. We also evaluate the infiuence of copy number of pCMV and tetO, in addition with concentration polymerase to the performance of this system respectively.

In part 2, we establish the model of doxycyclin-induced mCherry expression system with feedback. We use 7 reactions to build the model, with time delay and make necessary simplifications. Then we propose two ODEs to characterize these reactions. The stimulation result suggests that in this inducible system with feedback, the responsive range of doxycycline is much wider, and the induction response is gentler. We also access the infiuence of hill coefficient, binding affinity and basal rate of synthesis of tetR to the performance of this system respectively.

In part 3, we model the heterogeneity of Dox-induced singl-cell expression in both feedback and no-feedback systems. We model the random copy number variance, random epigenetic inheritance and random initial concentration of molecules in the population of single cells. For each doxycycline concentration, we stimulate the reaction for 1000 cells. The results of both models are compared and discussed.

In discussion, we compare the results of our modeling with the experiment results. We also discuss their similarities and differences. We also compare the results between system with feedback and system without feedback, and we discuss their similarities and differences.

Contents

1 Modeling doxycycline-induced GFP expression systems, without feedback

1.1 Informations

1. pCMV is a constitutive promoter.

2. Black triangle is a tetR-dimer binding site (tetO), there are two of them near TATA box.

3. NLS is nuclear localization sequence, facilitate the translocation of tetR to nucleus.

4. In the absence of Doxcycline, tetR-dimer preferably binds to tetO, block the RNA polymerase.

5. In the presence of Doxcycline, tetR-dimer preferably disassociate from tetO, remove the interference to RNA polymerase.

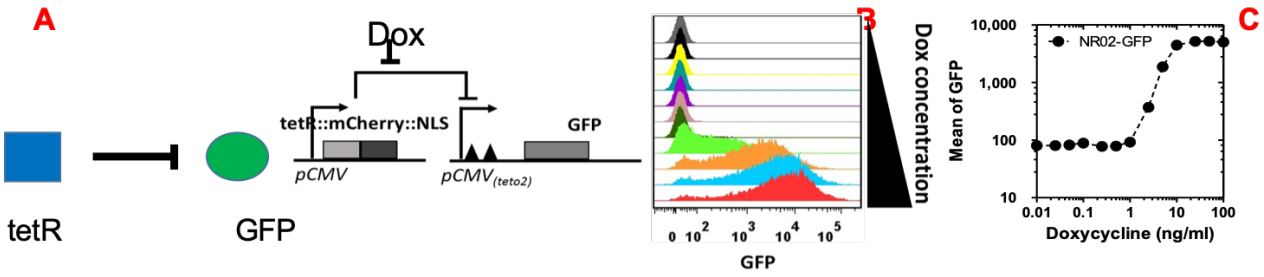

FIGURE 1.1: Pictures of part 1

1.2 Write step-by-step reactions

1.2.1 Symbols

TABLE 1.1: The symbols used in the model of doxycycline-induced GFP expression systems without feedback

Symbol	Definition
D	The doxycycline molecular
R	The tetR molecular
R_i^2	The tetR-dimer molecular binds with **i** doxycycline molecular
R_{sum}	Including R_0^2, R_1^2, R_2^2, R
O_m	The $tetO^2$ binding with m $R_0^2 (m = 0, 1, 2)$
O_{on}	The binding situation of $tetO^2$ that turn on the expression of GFP mRNA
O_{sum}	Including O_0, O_1, O_2
G	GFP
P	RNA polymerase
K_1	Reaction equilibrium constant of R_0^2 formation from R
K_2	Reaction equilibrium constant of R_1^2 formation from R_0^2
σ_1	$K_3 = \sigma_1 K_2$, so σ_1 represent binding strengths relative to the tetR-dimer-$tetO^2$ strength
K_3	Reaction equilibrium constant of R_2^2 formation from R_1^2
K_4	Reaction equilibrium constant of O_1 formation from O_0
σ_2	$K_5 = \sigma_2 K_4$, so σ_2 represent binding strengths relative to the tetR-dimer-doxycycline strength
K_5	Reaction equilibrium constant of O_2 formation from O_1
k_t	Reaction constant of GFP production
k_d	Reaction constant of protein degradation
P_0	I assume that the concentration of P remains constant as P_0 during time
n	The number of proteins per mRNA transcript
r	The basal rate of production of GFP

1.2.2 Model

The chemical reactions describing the network are naturally divided into two categories—fast and slow. The fast reactions have rate constants of order seconds and are therefore assumed to be in equilibrium with respect to the slow reactions, which are described by rates of order minutes.

Considering the reactions between tetR molecular, tetR-dimer molecular and doxycycline molecular, then we may write the equilibrium fast reactions :

$$\begin{aligned} 2R &\overset{K_1}{\rightleftharpoons} R_0^2 \\ R_0^2 + D &\overset{K_2}{\rightleftharpoons} R_1^2 \\ R_1^2 + D &\overset{K_3}{\rightleftharpoons} R_2^2 \end{aligned} \tag{1.1}$$

Considering the reactions between tetR-dimer molecular and $tetO^2$, and it is assumed that the tetR-dimer binding with doxycycline molecular could not binding to $tetO^2$, then we may write the equilibrium fast reactions :

$$\begin{aligned} O_0 + R_0^2 &\overset{K_4}{\rightleftharpoons} O_1 \\ O_1 + R_0^2 &\overset{K_5}{\rightleftharpoons} O_2 \end{aligned} \tag{1.2}$$

It is assumed that only O_0 can bind to $tetO^2$.
The slow reactions are transcription and degradation :

$$\begin{aligned} [O_{on}] &= [O_0] \\ O_{on} + P &\overset{k_t}{\rightarrow} O_{on} + P + nG \\ G &\overset{k_d}{\rightarrow} \phi \end{aligned} \tag{1.3}$$

I consider the expression level of tetR in the cell is stable, so $R_{sum} = 2([R_0^2] + [R_1^2] + [R_2^2]) + [R]$ is a constant value. Also, the number of $tetO^2$ is depended by the number of plasmids in the cell. So the number of $tetO^2$ is a constant, which means $O_{sum} = [O_0] + [O_1] + [O_2]$ does not change in a specific cell.

1.2.3 Reactions

$$2R \overset{K_1}{\rightleftharpoons} R_0^2$$

$$R_0^2 + D \overset{K_2}{\rightleftharpoons} R_1^2$$

$$R_1^2 + D \overset{K_3}{\rightleftharpoons} R_2^2$$

$$O_0 + R_0^2 \overset{K_4}{\rightleftharpoons} O_1$$

$$O_1 + R_0^2 \overset{K_5}{\rightleftharpoons} O_2$$

$$[O_{on}] = [O_0]$$

$$O_{on} + P \overset{k_t}{\rightarrow} O_{on} + P + nG$$

$$G \overset{k_d}{\rightarrow} \phi$$

1.3 Write ODE equations from reactions

Form the reactions above, ODE equations could be written and we know that :

$$\begin{aligned}
K_i &= \frac{k_i}{k_{-i}} (i = 1, 2, 3, 4, 5) \\
\frac{d[R]}{dt} &= 2k_{-1}[R_0^2] - 2k_1[R]^2 \\
\frac{d[R_0^2]}{dt} &= k_1[R]^2 + k_{-2}[R_1^2] + k_{-4}[O_1] + k_{-5}[O_2] \\
&\quad - (k_{-1} + k_2[D] + k_4[O_0] + k_5[O_1])[R_0^2] \\
\frac{d[R_1^2]}{dt} &= k_{-3}[R_2^2] - k_3[R_1^2][D] + k_2[R_0^2][D] - k_{-2}[R_1^2] \\
\frac{d[R_2^2]}{dt} &= k_3[R_2^1][D] - k_{-3}[R_2^2] \\
\frac{d[O_0]}{dt} &= k_{-4}[O_1] - k_4[O_0][R_0^2] \\
\frac{d[O_1]}{dt} &= k_4[O_0][R_0^2] - k_{-4}[O_1] + k_{-5}[O_2] - k_5[O_1][R_0^2] \\
\frac{d[O_2]}{dt} &= k_5[O_1][R_0^2] - k_{-5}[O_2] \\
\frac{d[G]}{dt} &= nk_t P_0[O_0] - k_d[G] + r
\end{aligned} \tag{1.4}$$

1.4 Simplified the model with quasi-steady state assumption and justified the assumptions

1.4.1 Simplification

Reaction in Eq.1.1 and Eq.1.2 can be simplified as steady-state. So I get the equations :

$$\begin{aligned}
[R_0^2] &= K_1[R]^2 \\
[R_1^2] &= K_2[D][R_0^2] \\
[R_2^2] &= K_3[D][R_1^2] \\
[O_1] &= K_4[R_0^2][O_0] \\
[O_2] &= K_5[R_0^2][O_1] \\
R_{sum} &= 2([R_0^2] + [R_1^2] + [R_2^2]) + [R] \\
O_{sum} &= [O_0] + [O_1] + [O_2] \\
K_3 &= \sigma_1 K_2 \\
K_5 &= \sigma_2 K_4
\end{aligned} \tag{1.5}$$

Considering that the formation of tetR-dimer is very fast, which means that the value of K_1 is large and $[R]$ is very small, we can have:

$$R_{sum} = [R_0^2] + [R_1^2] + [R_2^2] \tag{1.6}$$

Then these equations could be written as:

$$\begin{aligned}
[R_1^2] &= K_2[D][R_0^2] \\
[R_2^2] &= \sigma_1 K_2^2[D]^2[R_0^2] \\
[O_1] &= K_4[R_0^2][O_0] \\
[O_2] &= \sigma_2 K_4^2[R_0^2]^2[O_0] \\
R_{sum} &= [R_0^2] + [R_1^2] + [R_2^2] \\
O_{sum} &= [O_0] + [O_1] + [O_2]
\end{aligned} \tag{1.7}$$

From Eq.1.3 we can get an ODE equation :

$$\frac{d[G]}{dt} = nk_t P_0[O_0] - k_d[G] + r$$

Let $\alpha = nk_tP_0O_{sum}$, $\beta = K_4R_{sum}$, $y = [G]$, $x = [D]$, $N = K_2[D]$, $M = \beta[R_0^2]$
then :

$$\begin{aligned} \frac{dy}{dt} &= \frac{\alpha}{1 + M + \sigma_2 M^2} - k_d y + r \\ M &= \frac{\beta}{1 + N + \sigma_1 N^2} \\ N &= K_2 x \end{aligned} \tag{1.8}$$

1.5 Play with the parameters to mimic data from Figure 1.1 C

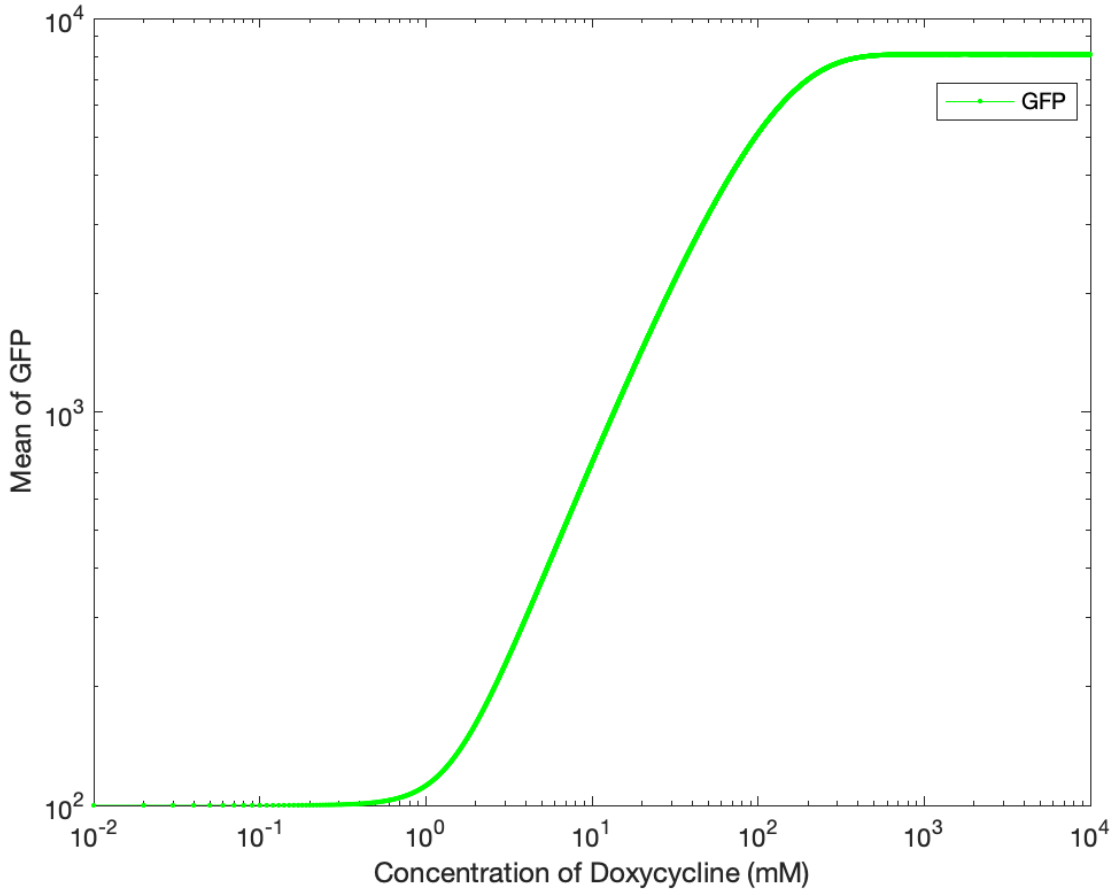

FIGURE 1.2: The modeling results for GFP with no feedback

$$K_2 = 100mM^{-1}; \sigma_1 = 0.5; \beta = 10000; \sigma_2 = 0.5;$$

$$\alpha = 80; kd = 0.01h^{-1}; r = 1mM * h^{-1}; y_0 = 100mM$$

2 Modeling doxycycline-induced mCherry expression systems, with feedback

2.1 Informations

1. pCMV is a constitutive promoter.

2. Black triangle is a tetR-dimer binding site (tetO), there are two of them near TATA box

3. NLS is nuclear localization sequence, facilitate the translocation of tetR to nucleus.

4. In the absence of Doxcycline, tetR-dimer preferably binds to tetO, block the RNA polymerase.

5. In the presence of Doxcycline, tetR-dimer preferably disassociate from tetO, remove the interference to RNA polymerase.

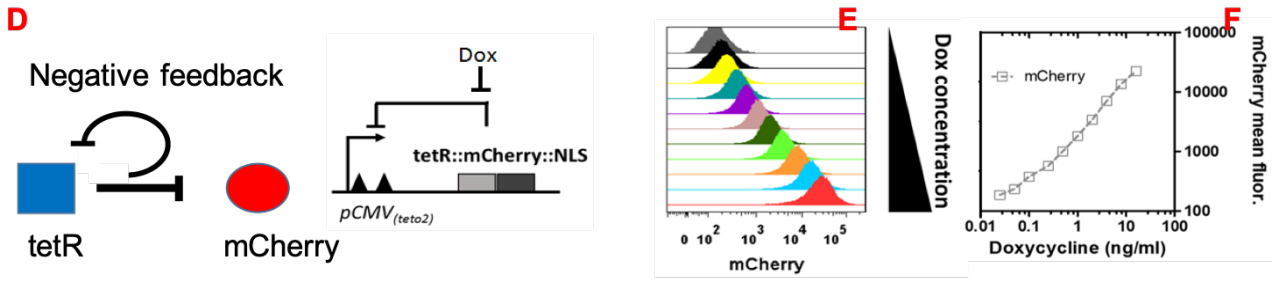

FIGURE 2.1: Pictures of part 2

2.2 Write step-by-step reactions

2.2.1 Symbols

TABLE 2.1: Modeling doxycycline-induced mCherry expression systems with feedback

Symbol	Definition
D	The doxycycline molecular
R	The tetR molecular
R_i^2	The tetR-dimer molecular binds with **i** doxycycline molecular
R^2	A mixture of R_0^2, R_1^2 and R_2^2
O_m	The $tetO^2$ binding with m $R_0^2 (m = 0, 1, 2)$
O_{on}	The binding situation of $tetO^2$ that turn on the expression of GFP mRNA
O_{sum}	Including O_0, O_1, O_2
C	mCherry protein
P	RNA polymerase
K_1	Reaction equilibrium constant of R_0^2 formation from R
K_2	Reaction equilibrium constant of R_1^2 formation from R_0^2
σ_1	$K_3 = \sigma_1 K_2$, so σ_1 represent binding strengths relative to the tetR-dimer-$tetO^2$ strength
K_3	Reaction equilibrium constant of R_2^2 formation from R_1^2
K_4	Reaction equilibrium constant of O_1 formation from O_0
σ_2	$K_5 = \sigma_2 K_4$, so σ_2 represent binding strengths relative to the tetR-dimer-doxycycline strength
K_5	Reaction equilibrium constant of O_2 formation from O_1
k_t	Reaction constant of mCherry and tetR production
k_{d-c}	Reaction constant of mCherry degradation
k_{d-rd}	Reaction constant of tetR-dimer degradation
P_0	I assume that the concentration of P remains constant as P_0 during time
n_c	The number of mCherry per mRNA transcript
n_{rd}	The number of tetR-dimer per mRNA transcript
r_c	The basal rate of production of mCherry
r_{rd}	The basal rate of production of tetR-dimer

2.2.2 Model

The chemical reactions describing the network are naturally divided into two categories—fast and slow. The fast reactions have rate constants of order seconds and are therefore assumed to be in equilibrium with respect to the slow reactions, which are described by rates of order minutes.

Considering the reactions between tetR molecular, tetR-dimer molecular and doxycycline molecular, then we may write the equilibrium fast reactions :

$$\begin{aligned} 2R &\overset{K_1}{\rightleftharpoons} R_0^2 \\ R_0^2 + D &\overset{K_2}{\rightleftharpoons} R_1^2 \\ R_1^2 + D &\overset{K_3}{\rightleftharpoons} R_2^2 \end{aligned} \tag{2.1}$$

If we ignore the exsit of tetR molecular and assume all of them will take part in the formation of tetR-dimer molecular, in aother word, the K_1 is extrimly huge, the reactions become :

$$\begin{aligned} R_0^2 + D &\overset{K_2}{\rightleftharpoons} R_1^2 \\ R_1^2 + D &\overset{K_3}{\rightleftharpoons} R_2^2 \end{aligned} \tag{2.2}$$

Considering the reactions between tetR-dimer molecular and $tetO^2$, and it is assumed that the tetR-dimer binding with doxycycline molecular could not binding to $tetO^2$, then we may write the equilibrium fast reactions :

$$\begin{aligned} O_0 + R_0^2 &\overset{K_4}{\rightleftharpoons} O_1 \\ O_1 + R_0^2 &\overset{K_5}{\rightleftharpoons} O_2 \end{aligned} \tag{2.3}$$

It is assumed that only O_0 can bind to $tetO^2$.
The slow reactions are transcription and degradation :

$$\begin{aligned} [O_{on}] &= [O_0] \\ O_{on} + P &\overset{k_t}{\rightarrow} O_{on} + P + n_c C + n_{rd} R^2 \\ C &\overset{k_{d-c}}{\rightarrow} \phi \\ R^2 &\overset{k_{d-rd}}{\rightarrow} \phi \end{aligned} \tag{2.4}$$

The number of $tetO^2$ is depended by the number of plasmids in the cell. So the number of $tetO^2$ is a constant, which means $O_{sum} = [O_0] + [O_1] + [O_2]$ does not change in a specific cell. Also, since the transcription reaction is slower than the reactions in Eq.2.1, the R_0^2 produced in transcription will partly become R_1^2 and R_2^2. So R^2 was used to donate this mixture of R_0^2, R_1^2 and R_2^2. $[R^2] = [R_0^2] + [R_1^2] + [R_2^2]$

2.2.3 Reactions

$$R_0^2 + D \overset{K_2}{\rightleftharpoons} R_1^2$$

$$R_1^2 + D \overset{K_3}{\rightleftharpoons} R_2^2$$

$$O_0 + R_0^2 \overset{K_4}{\rightleftharpoons} O_1$$

$$O_1 + R_0^2 \overset{K_5}{\rightleftharpoons} O_2$$

$$[O_{on}] = [O_0]$$

$$O_{on} + P \overset{k_t}{\rightarrow} O_{on} + P + n_c C + n_{rd} R^2$$

$$C \overset{k_{d-c}}{\rightarrow} \phi$$

$$R^2 \overset{k_{d-rd}}{\rightarrow} \phi$$

2.3 Write ODE equations from reactions

Form the reactions above, ODE equations could be written and we know that :

$$
\begin{aligned}
K_i &= \frac{k_i}{k_{-i}} (i = 2,3,4,5) \\
\frac{d[R_0^2]}{dt} &= +k_{-2}[R_1^2] + k_{-4}[O_1] + k_{-5}[O_2] \\
&\quad - (k_2[D] + k_4[O_0] + k_5[O_1])[R_0^2] \\
\frac{d[R_1^2]}{dt} &= k_{-3}[R_2^2] - k_3[R_1^2][D] + k_2[R_0^2][D] - k_{-2}[R_1^2] \\
\frac{d[R_2^2]}{dt} &= k_3[R_2^1][D] - k_{-3}[R_2^2] \\
\frac{d[O_0]}{dt} &= k_{-4}[O_1] - k_4[O_0][R_0^2] \\
\frac{d[O_1]}{dt} &= k_4[O_0][R_0^2] - k_{-4}[O_1] + k_{-5}[O_2] - k_5[O_1][R_0^2] \\
\frac{d[O_2]}{dt} &= k_5[O_1][R_0^2] - k_{-5}[O_2] \\
\frac{d[C]}{dt} &= n_c k_t P_0[O_0] - k_{d-c}[C] + r_c \\
\frac{d[R^2]}{dt} &= n_{rd} k_t P_0[O_0] - k_{d-rd}[R^2] + r_{rd} \\
[R^2] &= [R_0^2] + [R_1^2] + [R_2^2]
\end{aligned}
\tag{2.5}
$$

2.4 Simplified the model with quasi-steady state assumption and justified the assumptions

2.4.1 Simplification

Reaction in Eq.2.2 and Eq.2.3 can be simplified as steady-state. So I get the equations :

$$\begin{aligned}
[R_1^2] &= K_2[D][R_0^2] \\
[R_2^2] &= K_3[D][R_1^2] \\
[O_1] &= K_4[R_0^2][O_0] \\
[O_2] &= K_5[R_0^2][O_1] \\
K_3 &= \sigma_1 K_2 \\
K_5 &= \sigma_2 K_4
\end{aligned} \tag{2.6}$$

Then these equations could be written as:

$$\begin{aligned}
[R_1^2] &= K_2[D][R_0^2] \\
[R_2^2] &= \sigma_1 K_2^2[D]^2[R_0^2] \\
[O_1] &= K_4[R_0^2][O_0] \\
[O_2] &= \sigma_2 K_4^2[R_0^2]^2[O_0] \\
O_{sum} &= [O_0] + [O_1] + [O_2]
\end{aligned} \tag{2.7}$$

From Eq.2.4 we can get ODE equations :

$$\begin{aligned}
\frac{d[C]}{dt} &= n_c k_t P_0[O_0] - k_{d-c}[C] + r_c \\
\frac{d[R^2]}{dt} &= n_{rd} k_t P_0[O_0] - k_{d-rd}[R^2] + r_{rd} \\
[R^2] &= [R_0^2] + [R_1^2] + [R_2^2]
\end{aligned} \tag{2.8}$$

Then these equations could be written as :

$$\begin{aligned}
\frac{d[C]}{dt} &= n_c k_t P_0[O_0] - k_{d-c}[C] + r_c \\
\frac{d[R_0^2]}{dt} &= \frac{n_{rd} k_t P_0[O_0]}{1 + K_2[D] + \sigma_1 K_2^2[D]^2} - k_{d-rd}[R_0^2] + \frac{r_{rd}}{1 + K_2[D] + \sigma_1 K_2^2[D]^2}
\end{aligned} \tag{2.9}$$

Using O_{sum} to present $[O_0]$:

$$\begin{aligned}\frac{d[C]}{dt} &= \frac{n_c k_t P_0 O_{sum}}{1 + K_4[R_0^2] + \sigma_2 K_4^2[R_0^2]^2} - k_{d-c}[C] + r_c \\ \frac{d[R_0^2]}{dt} &= \frac{n_{rd} k_t P_0 O_{sum}}{(1 + K_2[D] + \sigma_1 K_2^2[D]^2)(1 + K_4[R_0^2] + \sigma_2 K_4^2[R_0^2]^2)} \\ &\quad - k_{d-rd}[R_0^2] + \frac{r_{rd}}{1 + K_2[D] + \sigma_1 K_2^2[D]^2}\end{aligned} \tag{2.10}$$

Then:

$$\begin{aligned}\alpha_1 &= n_c k_t P_0 O_{sum} \\ \alpha_2 &= n_{rd} k_t P_0 O_{sum} \\ x &= [D] \\ y &= [C] \\ N &= K_2 x \\ M &= 1 + N + \sigma_1 N^2 \\ U &= K_4[R_0^2] \\ V &= 1 + U + \sigma_2 U^2 \\ \frac{dy}{dt} &= \frac{\alpha_1}{V} - k_{d-c} y + r_c \\ \frac{d[R_0^2]}{dt} &= \frac{\alpha_2}{MV} - k_{d-rd}[R_0^2] + \frac{r_{rd}}{M}\end{aligned} \tag{2.11}$$

2.5 Play with the parameters to mimic data from Figure 2.1 F

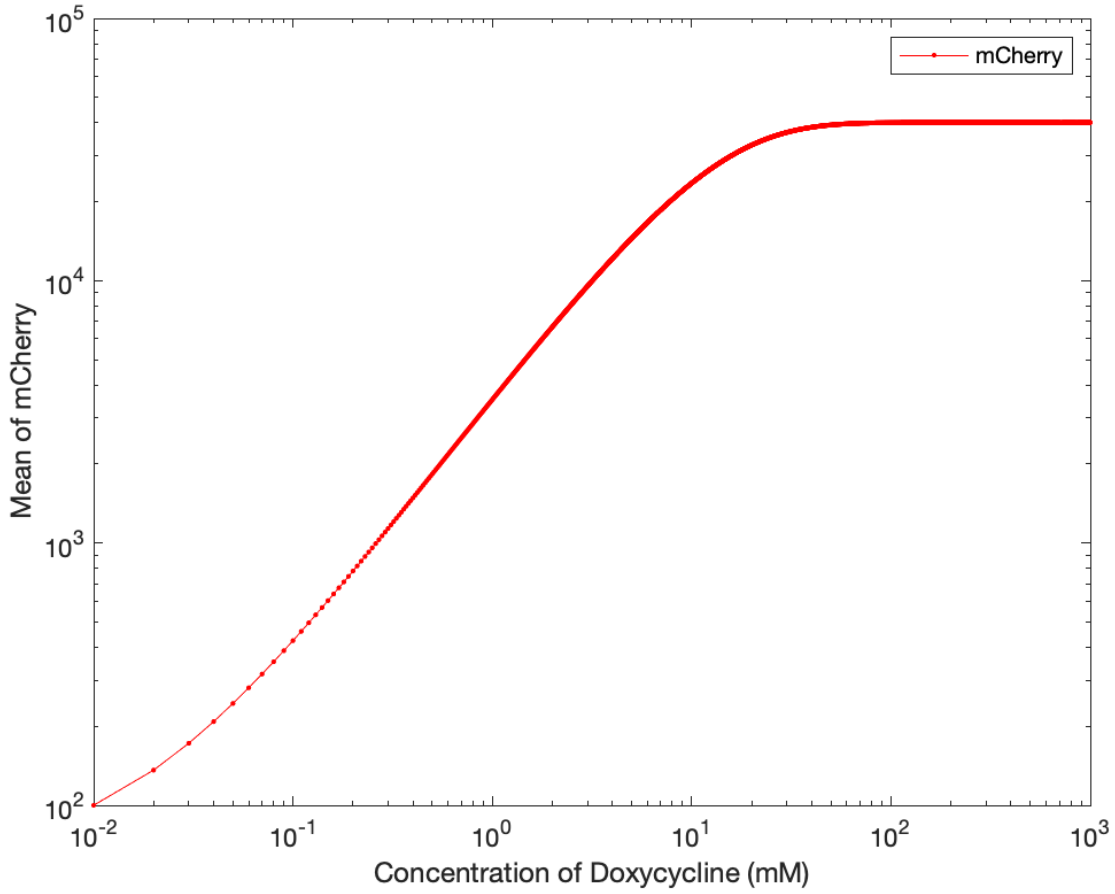

FIGURE 2.2: The modeling results for mCherry with feedback

$$K_2 = 1000mM^{-1}; \sigma_1 = 0.5; K_4 = 0.001mM^{-1}; \sigma_2 = 0.5; y_0 = 100mM; [R_0^2]_0 = 1000mM$$

$$\alpha_1 = 4000; \alpha_2 = 4000; kdc = 0.01h^{-1}; kdrd = 0.005h^{-1}; rc = 10mM * h^{-1}; rrd = 10mM * h^{-1}$$

2.6 Discuss the differences between Figures 2.1 F and 1.1 C, and explain them using the model

Both the fiuorescence of GFP with no feedback and the fiuorescence of mCherry with feedback are increased when the concentration of Doxycycline increased. But the processes are quite different.

For the modeling results for GFP with no feedback (shown as Figure 1.2), The slope of curve is changed, it is larger when the Dox concentration is near $1mM$ while is smaller in two side. Without feedback, the increasing GFP expression does not infiuencing the system itself. So that there is a threshold for concentration of Dox, when it reaches the threshold, the expression level of the target increases rapidly until the limit is reached.

For the modeling results for mCherry with feedback(shown as Figure 2.2), The slope of the curve is nearly unchanged, so it seems to be a line, which means the expression of mCherry is much more relates to the concentration of Dox. With feedback, the infiuence of dox is sensed, and the increasing tetR expression inhibits its own expression by binding to the promoter. The expression of the mCherry increases slowly, due to the suppression, and it's more stable and was directly changed by Dox.

3 Modeling the heterogeneity of the Dox-induced GFP (or mCherry) expressions

3.1 The model

In order to access the heterogeneity of Dox-induced single-cell expressions in both feedback and no-feedback systems, we model the random fiuctuation in biochemical reactions, random copy number variation and random epigenetic inheritance in expression systems.

3.1.1 Random epigenetic inheritance

Random epigenetic inheritance also plays an important part of fiuctuation in cell population. I incorporate random epigenetic inheritance on single cell level. Epigenetic regulations, including DNA methylation, histone modification and others, infiuence gene expression by regulating chromatin accessibility and the interaction between DNA and protein. In the model, the reaction constants of each transcription was influenced by a random variable μ which subscribes to normal distribution.

Where:

$$\begin{aligned} K_{real} &= K_{deterministic} \times (1 + \mu_1) \\ k_{d-real} &= k_{d-deterministic} \times (1 + \mu_2) \\ r_{real} &= r_{deterministic} \times (1 + \mu_3) \\ \beta_{real} &= \beta_{deterministic} \times (1 + \mu_4) \end{aligned} \tag{3.1}$$

3.1.2 Random copy number variance

Random copy number variance is an important source of noise in cell population. We incorporate random copy number variation on single cell level. In the modeling, the copy number

was considered in the α, which was influenced by a random variable τ which subscribes to normal distribution.

Where:

$$\alpha_{real} = \alpha_{deterministic} \times (1 + \tau) \tag{3.2}$$

3.1.3 Random basal expression level of GFP, mCherry and tetR-dimer

Random copy number variance is an important source of noise in cell population. We incorporate random copy number variation on single cell level. In the modeling, the basal expression level of GFP, mCherry and free tetR-dimer were influenced by a random variable ξ which subscribes to normal distribution.

Where:

$$\begin{aligned} [GFP]_{0-real} &= [GFP]_{0-deterministic} \times (1 + \xi_1) \\ [mCherry]_{0-real} &= [mCherry]_{0-deterministic} \times (1 + \xi_2) \\ [R_0^2]_{0-real} &= [R_0^2]_{0-deterministic} \times (1 + \xi_3) \end{aligned} \tag{3.3}$$

3.2 Simulate the flow cytometry data with simulations of 1000 cells

Combing the factors of random fiuctuation in biochemical reactions, random copy number variance and random epigenetic inheritance described above, we establish model to study the heterogeneity of Dox-induced single-cell expressions in both feedback and no-feedback systems. For each doxycycline concentration, we stimulate 1000 single cells.

3.2.1 Heterogeneity of Dox-induced single-cell expressions, without feedback

For the heterogeneity of Dox-induced single-cell expressions of GFP without feedback, the stimulation result is as follows:

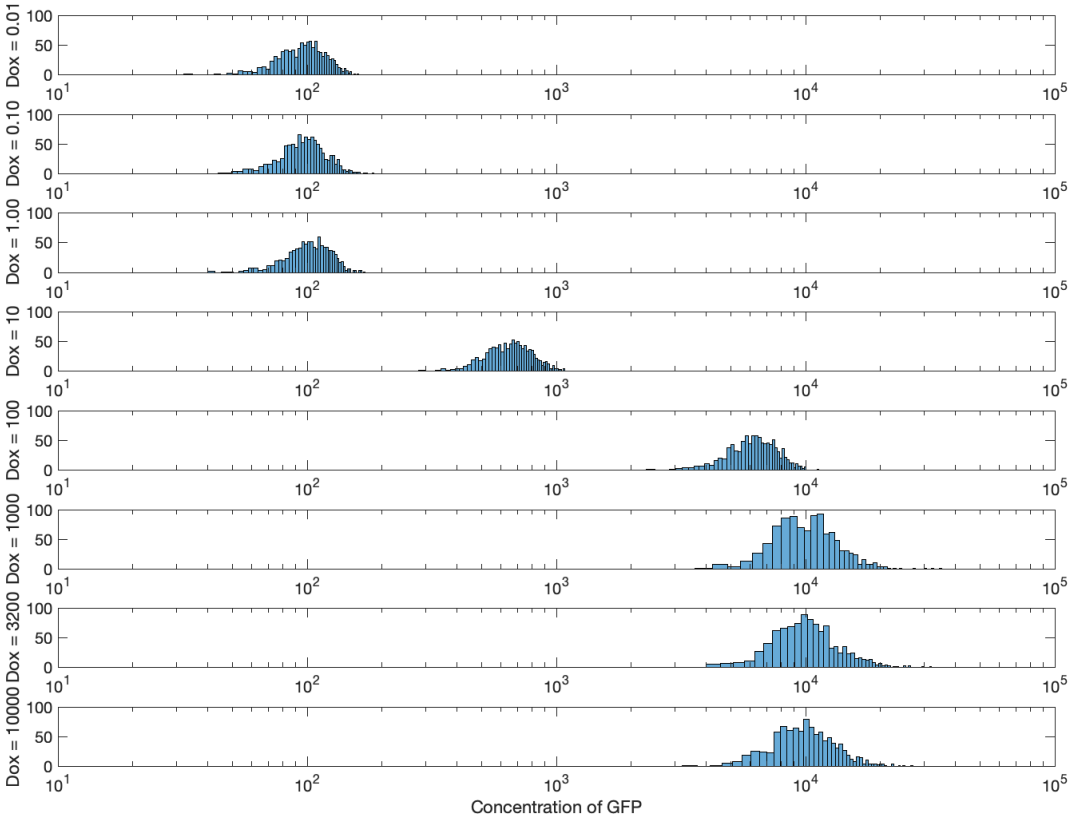

FIGURE 3.1: Cytometry simulations of Dox-induced GFP expression system without feedback in 1000 cells

From the figure we can see that for each concentration of doxycycline, the GFP expression in cell population is like a normal distribution. At low concentration of doxycycline, increasing doxycycline concentration would not cause much to distribution of GFP expression, this is because at this stage there is surplus of tetR dimer in addition to binding most of tetO. At higher concentration of doxycycline, increasing doxycycline concentration would not cause much change to the distribution of GFP expression, this is because at this stage the doxycycline is enough to bind most of the tetR dimer.

3.2.2 Heterogeneity of Dox-induced single-cell expressions, with feedback

For the heterogeneity of Dox-induced single-cell expressions of mCherry with feedback, the result is as follows:

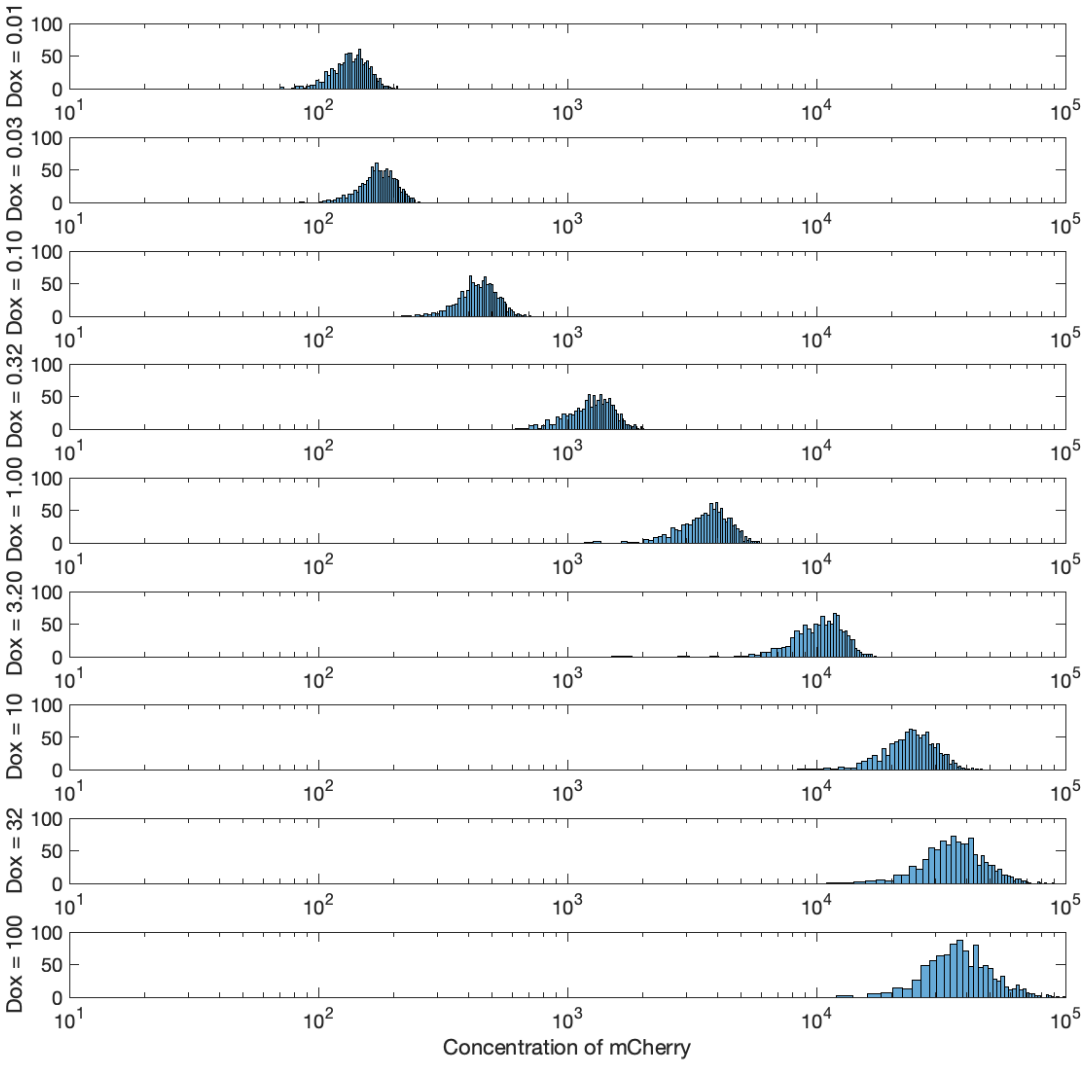

FIGURE 3.2: Cytometry simulations of Dox-induced mCherry expression system with feedback in 1000 cells ($log10 \approx 3.2$)

From the figure we can see that for each concentration of doxycycline, the mCherry expression in cell population is like a normal distribution. As the concentration of doxycycline increase, the mean of mCherry expression show a steady increase. This is because there is a negative feedback control loop in this system, increasing the concentration of doxycycline will always lead to the system to reach a new equilibrium.

3.3 Discussion

In this session, we discuss the result and compare our results with the experiment result.

For part1, our result looks very similar to the experiment result, including the initial value, the end value and the turning point. At low concentration of doxycycline, increasing doxycycline concentration would not cause much difference to GFP expression, this is because at this stage there is surplus of tetR dimer in addition to binding most of tetO. At higher concentration of doxycycline, increasing doxycycline concentration would not cause much to GFP expression too, this is because at this stage the doxycycline is enough to bind most of the tetR dimer. This system is responsive to doxycycline only in a narrow range of doxycycline concentration, and the response is ultrasensitive.

For part2, our result also looks very alike to the experiment result, including the initial value and the end value. As the concentration of doxycycline increase, the mean of mCherry expression show a steady increase. This is because there is a negative feedback control loop in this system, increasing the concentration of doxycycline will always lead to the system to reach a new equilibrium. This system is sensitive to a wide range doxycycline and it is easy to tune to achieve precise and quantitate control mCherry expression.

We combine the result of part1 and part2 together, the result is as follows:

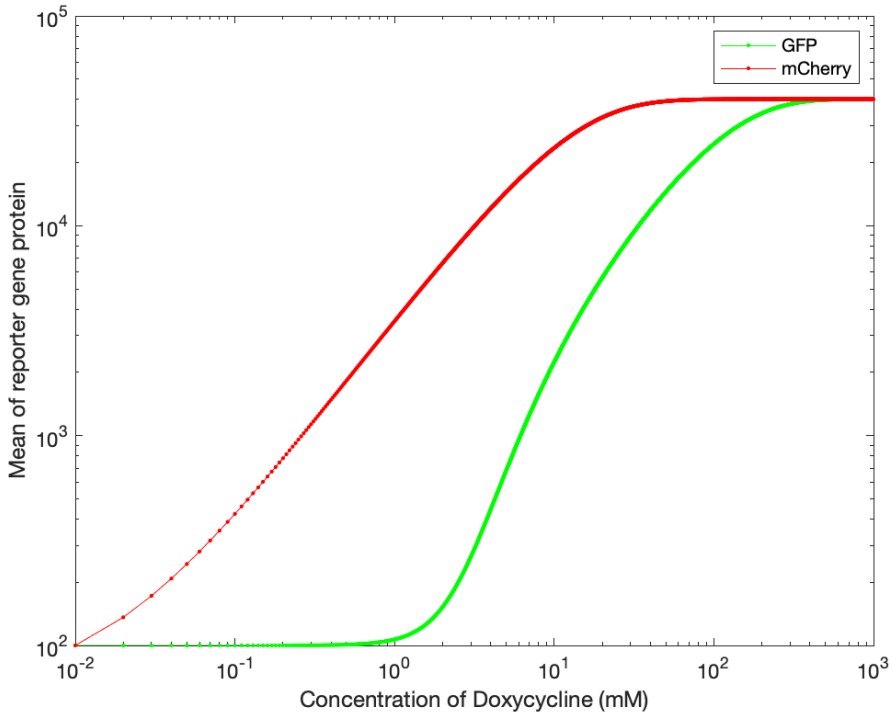

FIGURE 3.3: The modeling results for GFP without feedback and mCherry with feedback

The system without feedback is ultrasensitive to doxycycline in a narrow concentration range, while the system with feedback is sensitive to a wide range doxycycline and it is easy to tune to achieve precise and quantitate control mCherry expression. The difference is due to the feedback control: It can control the equilibrium of the reaction and stabilize the system, thus the system with feedback is responsive to wider range of doxycycline concentration, and increasing the concentration of doxycycline will lead to gentle change in target gene expression.

For part3, our modeling result of the system without feedback has similar mean value of the distribution comparing with the experiment result. But at higher concentration of doxycycline, the distribution of experiment result has heavier tail than our modeling result. We hypothesis that the difference is due to some unknown biological pathways. For instance, at higher concentration of doxycycline, some other biological pathways are affected thus lead to the heavier tail in the distribution of experiment result. In addition, our modeling result of system with feedback is similar to the experiment result, both in mean value and distribution shape.