CHAPTER 3: RESULTS

3.1 Dynamics of C2-FFL with AND/OR gate

Figure 3.1 shows the dynamics for C2-FFL with AND gate. At the ON step, transcription factor X is activated by its inducer, causing the concentration of its active form $X^*$ to increase. Protein Y and Z begin to decrease when $X^*$ exceeds $K_{xy}$ and $K_{xz}$. At the OFF step, the activation signal for protein X is removed. When $X^*$ falls below $K_{xy}$ and $K_{xz}$, the production of protein Y resumes. In C2-FFL with AND gate, the production of protein Z requires the concentration of active form of protein Y exceeding $K_{yz}$ and the concentration of active form of X falling below $K_{xz}$. Only when this condition is achieved will the production of protein Z begin. This explains the delay in activation of protein Z production. The delay lengthens the response time (time taken to reach half of the steady-state concentration) of C2-FFL.
Figure 3.1: Dynamics of C2-FFL with AND gate at ON and OFF step.

In C2-FFL with OR gate (Figure 3.2) at the ON step, protein Z will be produced as long as the active form of Y exceeds $K_{Yc}$. When protein X is activated by its inducer and the concentration of its active form $X^*$ reaches $K_{xy}$ and $K_{xz}$, the production of protein Y is repressed. The concentration of protein Z remains unaffected for a while because the amount of Y is still sufficient to maintain its production. When the concentration of protein Y falls below $K_{Yc}$, the production of protein Z ceases, causing its concentration to decrease.
Thus, there is a short delay in repression of protein Z production. At the OFF step, once the concentration of $X^*$ falls below $K_{xz}$ and $K_{xy}$, it is not able to repress the production of protein $Y$ and $Z$, and basically the network degenerates into simple regulation of $Z$ by $Y$. There is only a short delay in the production of protein $Z$, with corresponding shorter response time.

Figure 3.2: Dynamics of C2-FFL with OR gate at ON and OFF step.
3.2 Dynamics of C3-FFL with AND/OR gate

In C3-FFL with AND gate, the production of protein $Z$ is activated in the absence of both $X^*$ and $Y^*$ (Figure 3.3). At the ON step, when $X^*$ exceeds $K_{xz}$, it results in immediate stoppage of the production of protein $Z$. Thus, the concentration of protein $Z$ decreases with its degradation/dilution rate and there is no delay of repression. Simultaneously, the production of protein $Y$ is activated by $X^*$ when $X^*$ exceeds $K_{xy}$.

At the OFF step of C3-FFL with AND gate, the activation signal for $X^*$ is switched off and $X^*$ decreases. When $X^*$ falls below $K_{xz}$ and $K_{xy}$, the production of protein $Y$ is inactivated and decreases while production of protein $Z$ is not resumed due to the presence of its repressor. Lately, when $Y^*$ falls below $K_{yz}$, protein $Z$ begins to be produced; production is delayed until both $X^*$ and $Y^*$ fall below $K_{xz}$ and $K_{yz}$, respectively.
Figure 3.3: Dynamics of C3-FFL with AND gate at ON and OFF step.

The dynamics of C3-FFL with OR gate is similar to the dynamics of C3-FFL with AND gate (Figure 3.4). At the ON step, the concentration of $X^*$ is sufficient enough to repress the production of protein Z regardless of the absence of $Y^*$. Thus, the production of protein Z is switched off without delay. At the OFF step, either $X^*$ or $Y^*$ falls below the repression threshold when the production of protein Z resumes. Thus, there is delay in resuming protein Z production.
3.3 Dynamics of C4-FFL with AND/OR gate

In C4-FFL with AND gate, the production of protein Z is activated in the presence of \( X^* \) and absence of \( Y^* \) (Figure 3.5). At the ON step, the concentration of \( X^* \) exceeds \( K_{xy} \) and results in cease of production of protein Y. To show the effect of repression of protein Z production by \( Y^* \), protein Z was given a starting concentration of 3.5. When the
concentration of $Y^*$ has not reduced below $K_{yz}$, the concentration of protein $Z$ decreases until concentration of $Y^*$ falls below the $K_{yz}$. In the absence of $Y^*$, the concentration of $X^*$, now above $K_{xz}$, is able to resume the production of protein $Z$ before it decreases to zero. Thus, there is delay in the production of protein $Z$.

At the OFF step of C4-FFL with AND gate, the activation signal for $X^*$ is switched off and $X^*$ decreases. When $X^*$ falls below $K_{xz}$ and $K_{xy}$, the production of protein $Y$ is resumed while production of protein $Z$ decreases. Lately, when $Y^*$ falls below $K_{yz}$, the production of protein $Z$ is delayed when both $X^*$ and $Y^*$ are absent. Thus, there is no delay of repression of protein $Z$ production.
Figure 3.5: Dynamics of C4-FFL with AND gate at ON and OFF step.

Figure 3.6 shows that the dynamics of C4-FFL with OR gate is similar to the dynamics of C4-FFL with AND gate. Similar to the case of the dynamics of C3-FFL with AND and OR gate, the Boolean input gate has no effect on the response time pattern in C4-FFL with the presence and absence of activation signal for protein X. Thus, there is delay of activation of protein Z production at the ON step and no delay of repression of protein Z production at the OFF step in C4-FFL.
3.4 Dynamics of I2-FFL with AND gate

In I2-FFL with AND gate, the production of protein $Z$ is activated when both $X^*$ and $Y^*$ are absent (Figure 3.7). At the ON step, $X^*$ exceeds $K_{xy}$ and $K_{xz}$, and results in the cease of the production of protein $Y$ and $Z$. Thus, there is no delay of repression of protein $Z$ production.

Figure 3.6: Dynamics of C4-FFL with OR gate at ON and OFF step.
At the OFF step of I2-FFL with AND gate, the activation signal for $X^*$ is switched off and $X^*$ decreases. It shows interesting dynamics pattern which is not found in all coherent FFL, the acceleration in production of protein Z at certain time point. When $X^*$ falls below the $K_{xz}$ and both $X^*$ and $Y^*$ are absent, the activation of protein Z production is enhanced and accelerated to the concentration higher than its steady-state. When the production of protein Y is resumed and exceeds $K_{yz}$, the production of protein Z is repressed and decreases to its steady-state. Thus, there is acceleration in protein Z production and its concentration remains at steady-state when it is repressed by $Y^*$. 
Figure 3.7: Dynamics of I2-FFL with AND gate at ON and OFF step.
3.5 Dynamics of I3-FFL with AND gate

In I3-FFL with AND gate, the production of protein Z is activated in the absence of \( X^* \) and in the presence of \( Y^* \) (Figure 3.8). At the ON step, \( X^* \) exceeds \( K_{xy} \) and \( K_{xz} \), and results in the cease of the production of protein Z without depending on the concentration of \( Y^* \). Thus, there is no delay of repression of protein Z production.

At the OFF step of I3-FFL with AND gate, the activation signal for \( X^* \) is switched off and \( X^* \) decreases. Despite the fact that there is reduction of response time in production of protein Z, its concentration during production time does not accelerate. Protein Y production is repressed when \( X^* \) falls below \( K_{xy} \).
Figure 3.8: Dynamics of I3-FFL with AND gate at ON and OFF step.

To investigate the dependency of gene Y promoter activity on accelerating pattern of protein Z production in I3-FFL with AND gate at the OFF step, the promoter activity of gene Y was given values of 5, 7.5 and 10 and their dynamics were simulated to obtain the production of protein Z at the OFF step with different gene Y promoter activity (Figure 3.9). The simulation result shows that there is acceleration in protein Z production when the gene
$Y$ promoter activity is the strongest at 10. Thus, it indicates that the strength of gene $Y$ promoter contributes to the accelerating pattern in protein $Z$ production at the OFF step.

**Figure 3.9:** The Effect of gene $Y$ promoter activity on acceleration of protein $Z$ production in I3-FFL with AND gate at OFF step.
3.6 Dynamics of I4-FFL with AND gate

The dynamics of I4-FFL with AND gate is opposite to the dynamics of I3-FFL with AND gate in term of the ON or OFF step. In I4-FFL with AND gate, the production of protein Z is activated in the presence of $X^*$ and $Y^*$ (Figure 3.10). The production of protein $Y$ is repressed by $X^*$. At the ON step, $X^*$ exceeds $K_{xz}$ and results in inactivation of protein Z production. When $Y^*$ does not fall below the $K_{yz}$, the production of protein Z is enhanced and the response time is shortened. Nevertheless, there is no acceleration observed in protein Z production.

At the OFF step of I4-FFL with AND gate, the activation signal for $X^*$ is switched off and $X^*$ decreases. At the ON step, $X^*$ falls below $K_{xy}$ and $K_{xz}$, and results in the resume of protein $Y$ production and the cease of the production of protein Z without depending on the concentration of $Y^*$. Thus, there is no delay of repression of protein Z production.
Figure 3.10: Dynamics of I4-FFL with AND gate at ON and OFF step.

To investigate the dependency of gene $Y$ promoter activity on accelerating pattern of protein $Z$ production in I4-FFL with AND gate at the ON step, the promoter activity of gene $Y$ was given values of 5, 7.5 and 10 and their dynamics were simulated to obtain the production of protein $Z$ at the OFF step with different gene $Y$ promoter activity (Figure 3.11). The simulation result shows that there is an acceleration of protein $Z$ production when the gene $Y$ promoter activity is at 7.5 or 10. Thus, similar to I3-FFL with AND gate at
the OFF step, it indicates that the strength of gene $Y$ promoter contributes to the accelerating pattern in protein $Z$ production at the ON step.

**Figure 3.11:** The Effect of gene $Y$ promoter activity on acceleration of protein $Z$ production in I4-FFL with AND gate at ON step.
3.7 Peculiar FFL transcription networks in *E. coli*

The result of mining the RegulonDB showed that 84 of 1702 TF-operon interactions were identified as being involved. In total, 28 FFL were identified in transcription networks of *E. coli*; nine of them were identified as belonging to peculiar FFL. No I2-FFL and I4-FFL were identified from the datasets of TF-operon interactions.

### Table 3.1: List of peculiar FFL associated with various biological functions extracted from TF-operon interactions, RegulonDB.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>FFL Type</th>
<th>Biological functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>crp</em></td>
<td><em>malI</em></td>
<td><em>malXY</em></td>
<td>C4-FFL</td>
<td>Maltose utilisation</td>
</tr>
<tr>
<td><em>fnr</em></td>
<td><em>arcA</em></td>
<td><em>cydAB</em></td>
<td>I3-FFL</td>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td><em>fnr</em></td>
<td><em>arcA</em></td>
<td><em>cyoABCDE</em></td>
<td>C3-FFL</td>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td><em>fnr</em></td>
<td><em>arcA</em></td>
<td><em>icdA</em></td>
<td>C3-FFL</td>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td><em>fnr</em></td>
<td><em>arcA</em></td>
<td><em>ndh</em></td>
<td>I3-FFL</td>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td><em>fnr</em></td>
<td><em>arcA</em></td>
<td><em>nuoABCEFGLHJKLMN</em></td>
<td>C3-FFL</td>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td><em>fnr</em></td>
<td><em>arcA</em></td>
<td><em>sdhCDAB_b0725_sucABCD</em></td>
<td>C3-FFL</td>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td><em>ihf</em></td>
<td><em>ompR_envZ (ompB)</em></td>
<td><em>ompC</em></td>
<td>C2-FFL</td>
<td>Osmoregulatory response</td>
</tr>
<tr>
<td><em>ihf</em></td>
<td><em>ompR_envZ (ompB)</em></td>
<td><em>ompF</em></td>
<td>C2-FFL</td>
<td>Osmoregulatory response</td>
</tr>
</tbody>
</table>