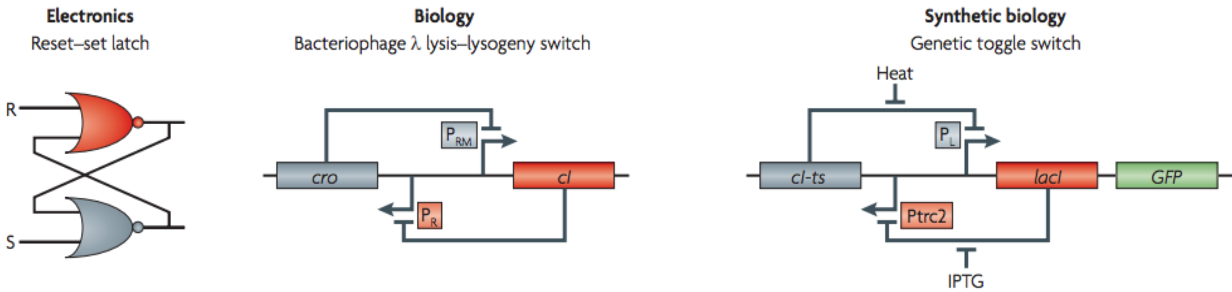
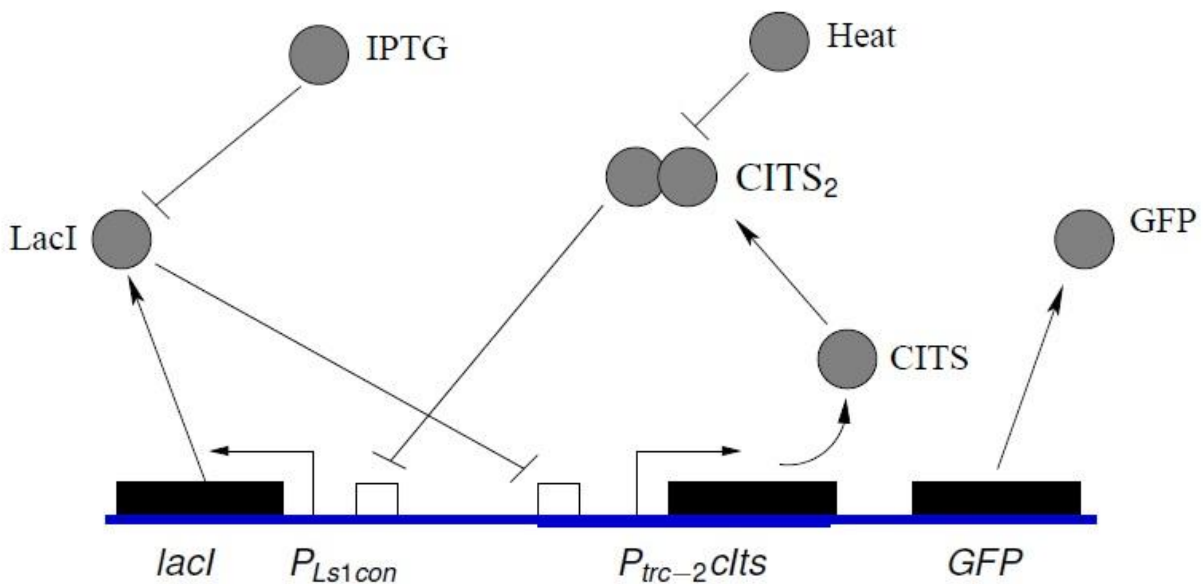
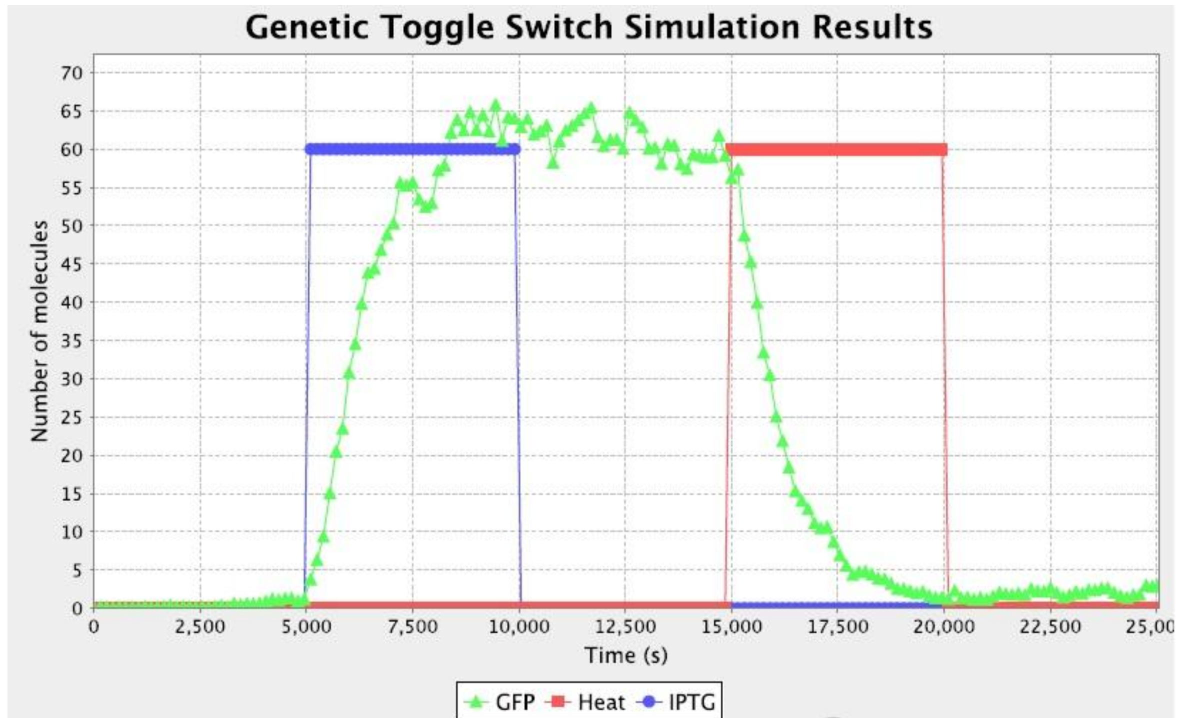
Modelling



Toggle switch is a gene synthetic network that was discovered and constructed in *Escherichia coli* by Tim Gerdner, Charles Cantor, and Jim Collins. Naturally, toggle switch gene synthetic network found in lambda bacteriophage for regulate its lytic and lysogenic cycle. In synthetic biology, toggle switch use for regulating bistability of two expression system by orientating to one expression system depends on strength of the promoter and inducer or repressor concentration.

Logic for toggle switch gene synthetic network was described in picture below.





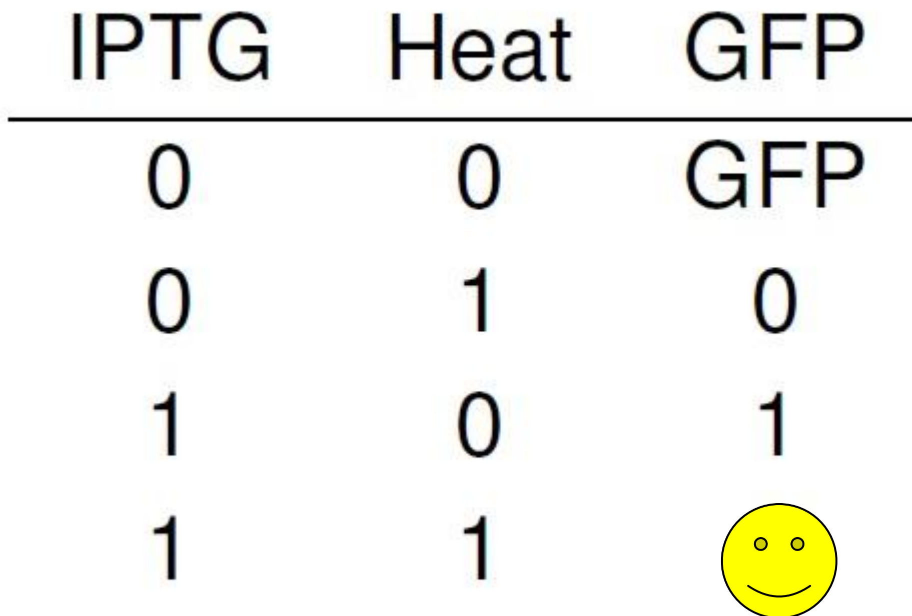
Based on picture above, when IPTG present, GFP will constitutively expressed even when IPTG no longer present or degraded. However, when heat present, GFP expression will stop and expression will convert to another expression orientation, in this case there is no protein expression because there is no gene in upstream of Pls1con.

In simulation graph above, not representing all possibilities of logic could occur in toggle switch yet. Logic that not explained yet is if IPTG and heat present. In toggle switch, when two inducer present, we couldn't determine a conservative result just like another genetic oscillator, because toggle switch expression orientation is depend on promoter strength and inducer or repressor concentration. Because of every toggle switch is different, determination of expression orientation of toggle switch when two inducer present should be done experimentally.

Toggle switch logic was simplified in table below.

IPTG	Heat	GFP
0	0	GFP
0	1	0
1	0	1
1	1	?

In our system, we use strong promoter, XylA promoter, to express subtilosin A and we use weaker promoter, hyper spank promoter, to stop subtilosin A expression or conducting cell death. So, hypothetically, when there are two inducer in our toggle switch gene synthetic network, expression of Subtilosin A will occur instead of stop.



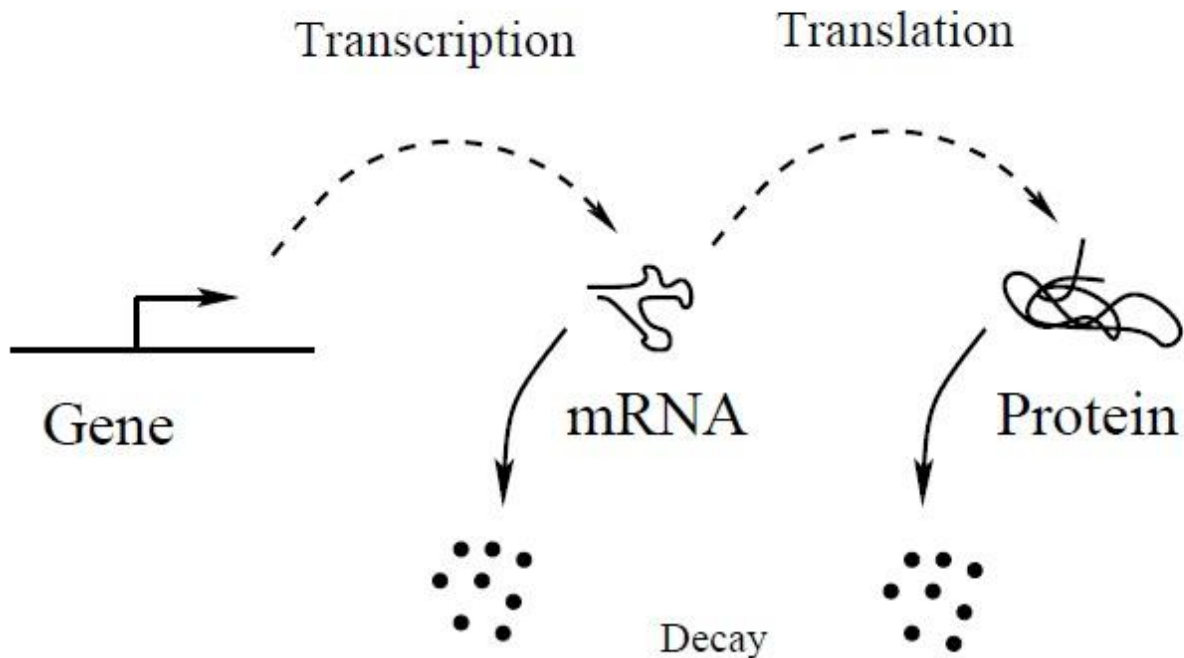
IPTG	Heat	GFP
0	0	GFP
0	1	0
1	0	1
1	1	

Mathematical Modeling

In synthetic biology, we usually use mass action kinetic differential equation to model expression system mathematically. Mass action kinetic says that reaction rate equal to reaction rate constant times reaction product mass.

In gene expression, mathematical modeling follow central dogma of biology, but we have to involve decay of messenger RNA and decay of protein.

Mathematical modeling of gene expression follow scheme below.

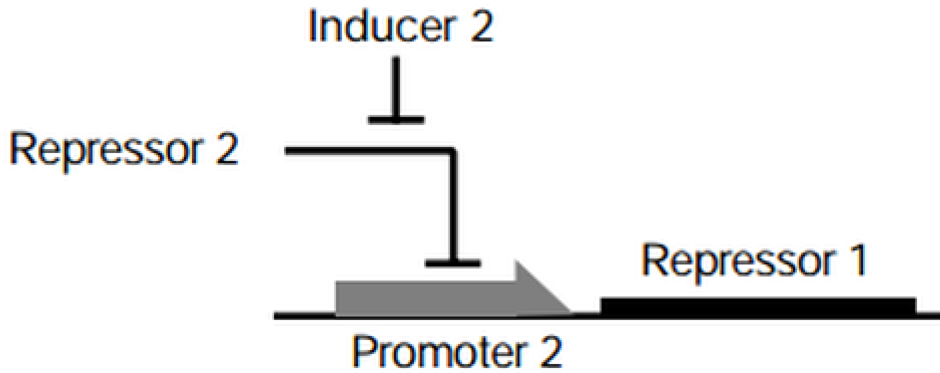


Mathematically, scheme above could be describe to equation below.

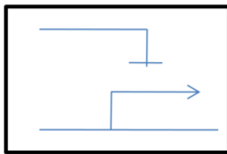
$$\frac{d[mRNA]}{dt} = k_{transcription}[DNA] - \gamma_m[mRNA]$$

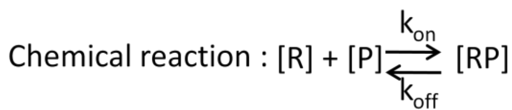
$$\frac{d[Protein]}{dt} = k_{translation}[mRNA] - \gamma_p[Protein]$$

In toggle switch, before we construct equation, we have to reduce toggle swtich complex into one expression system indepently.



Production of mRNA depends on transcription constant and activator or repressor concentration.





[R} = free Repressor
[P] = free Promoter*
[RP] = Repressor-Promoter complex

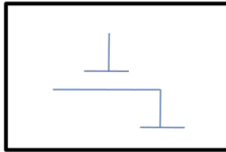
Bound fraction = $\left(\dfrac{K[R]}{1+K[R]}\right)^n$

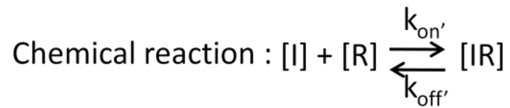
$K = k_{on} / k_{off}$
n = Hill coefficient

In repression model, we use :
Unbound fraction = 1 – bound fraction = $\left(\dfrac{1}{1+K[R]}\right)^n$

Promoter activity = $K_{transcription}$. Unbound fraction = $\dfrac{Ktranscription}{1+(K[R])^n}$

In toggle switch, promoter activity also depends on inducer concentration because inducer inhibit repressor to repress promoter.



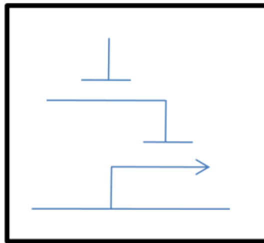


Chemical reaction : $[I] + [R] \underset{k_{off'}}{\overset{k_{on'}}{\rightleftharpoons}} [IR]$

[R} = free Repressor
[I] = free Inducer
[IR] = Inducer-Repressor complex

Unbound fraction = $\left(\frac{1}{1 + K'[I]}\right)^{n'}$ = $\frac{[R]}{[IR] + [R]}$

$$[R] = \frac{[IR]}{(K'[I])^{n'}}$$



$$\frac{d[mRNA]}{dt} = k_{transcription}[DNA] - \gamma_m[mRNA]$$

Promoter Activity = $\frac{Ktranscription}{1 + (K[R])^n}$

$$[R] = \frac{[IR]}{(K'[I])^{n'}}$$

$$\frac{d[mRNA]}{dt} = \frac{Ktranscription}{1 + K\left(\frac{[IR]}{(K'[I])^{n'}}\right)^{n}} - \gamma m[mRNA]$$

For protein production rate, we have to multiply transcription rate with number of molecules of protein product.

$$protein\ production\ rate = P.\frac{Ktranscription}{1 + K\left(\frac{[IR]}{(K'[I])^{n'}}\right)^{n}}$$

P = Number of molecule of protein product

Degradation time of protein depends on concentration of protein and its lifetime.

$$protein\ degradation\ rate = \frac{[protein]}{T}$$

T = protein lifetime

Using two equations above, we could generate differential equation of protein product as a function of time.

$$\frac{d[Protein]}{dt} = P.\frac{Ktranscription}{1 + K\left(\frac{[IR]}{(K'[I])^{n}}\right)^{n'}} - \frac{[Protein\}}{T}$$