4.1 The Kinetic Approach

In our previous model, we have assumed LacI, AraC, and many other species to be in fast thermodynamic equilibrium, and although Hill's Equation and Michaelis-Menten equation has been invoked to better depict the dynamic changes of certain biochemical processes, we are aware of the potential restraints Hill's Equation and Michaelis-Menten equation's approximations may have on the accuracy of our model.

Admittedly, viewed from the timescale of our experiment operations, the concentration of various species will "quickly" reach equilibrium. These biochemical processes involve molecules that interact with each other at a much slower rate comparing to small inorganic molecules whose reactions could be properly depicted with fast-equilibriums. As a result, the factor of time shouldn't be neglected in our modeling.

We decided to take the kinetic approach-all the biochemical processes presented are described with components of the rate law equations. The dynamic change of the concentration of each species will be described by a differential equation. A solvable system of n differential equations with n variables could be obtained from each differential equation. We can solve for the change of concentrations of all species considered according to time in this way.

Although the kinetic approach seems to be an ideal method, our implementation of the kinetic approach is far from perfect. When applying rate laws, we assumed many reactions to be elementary reactions or to be irreversible to simplify our calculations. This simplification could result in errors if the mechanism of actual biological processes differs significantly

from our assumption. Further investigations into the mechanism of these biochemical processes are needed to thoroughly reveal the true strength of the kinetic approach and its advantages over the thermodynamic approach.

4.2 Concentrations, Counts, and the "Death" Line

We describe promoters on DNA and proteins with their respective concentrations instead of their numbers. Some may question our use of concentrations instead of counts since some biological molecules exist in very small numbers inside bacteria. However, we can prove our approach to be reasonable with some crude calculations:

Consider an *E Coli*. with a volume of $0.7\,\mu m = 0.7\times10^{-15}\,L$ that contains one plasmid. The concentration of the plasmid will be $1 \div N_A \div 0.7\times10^{-15}\,L \approx 2.37\times10^{-9}\,M$. The concentration is significant enough for us to operate with.

In the meantime, the bulk of the kinetic theory is developed based on using concentration to describe dynamic changes of reactions, and therefore it would be easier for us to implement the kinetic approach using concentration instead of counts.

Nevertheless, our preference to use concentrations doesn't undermine the importance of counts. Counts matter not only due to its correlation with concentration concluded in $Concentration = \frac{Counts}{N_A \cdot Volume}$, but also in the constraint it has on concentrations—there is only positive integers of counts, or in other words, $counts \geq 1$. This obvious statement has actually

posed a condition to concentrations that shouldn't be overlooked—

$Concentration \geq \frac{1}{N_A \times Volume}$.

We refer to $\frac{1}{N_A \times Volume}$ as the "death" line, since a concentration lower than this means that this kind of molecule has a count smaller than 1, and therefore doesn't exist and should have a concentration equals to zero.

4.3 Stereochemistry of Proteins and Protein Complexes

Another key highlight of this model is the consideration of the stereochemistry of proteins and protein complexes. We first started to consider stereochemistry when we are trying to handle the LacI-iPTG system.

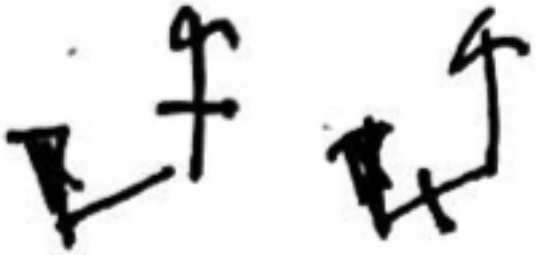
As mentioned before, LacI could form dimers denoted as $LacI_2$ as well as tetramers denoted as $LacI_4$. Both of these species could interact with iPTG. Each LacI has one and only one binding site for iPTG, and LacI polymerization doesn't create or eliminate iPTG binding sites. Therefore, each $LacI_2$ has 2 iPTG binding sites, and each $LacI_4$ has 4 iPTG binding sites.

LacI only has one way to bind iPTG since it only has one binding site. A LacI with one iPTG is denoted as LacI-1. $LacI_2$ and $LacI_4$ only have one way to bind with two and four iPTGs respectively since their limited capacities indicate that at full iPTG-saturated mode, they could only go with "all-full".

Each $LacI_2$ will form one kind of product from binding one iPTG if two of its bind sites are completely identical. Nevertheless, after thorough investigations, we realized that $LacI_2$ should be considered to be chiral and directional, as represented in our following graph.

To emphasize the directionality of the dimer, the arrow represents the "head" of it. To emphasize its dimensionality, a wedge (a common notation in organic chemistry representing bonds extending towards viewers) is added at its tail.

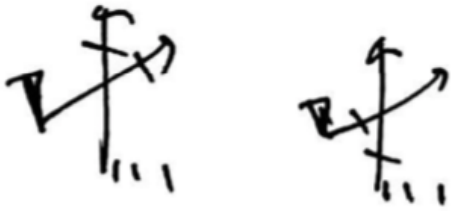
$LacI_2$ is capable of forming two kinds of $LacI_2$+single iPTG complexes as shown here, with the dash crossing the previous figure representing iPTG. The complex on the left is represented as $LacI_2$-1H, with H representing head as its "head-end" combines an iPTG, and the complex on the right is represented as $LacI_2$-1T, with T representing tail as its "tail end" combines with an iPTG.



For $LacI_4$, we believe that it's best to represent it as two dimers combined back to back with heads pointing in the same direction. To combine them back to back, one dimer has to be flipped horizontally, and therefore the wedge turns into a dash (a common notation in organic chemistry representing bonds extending away from viewers).

Unsurprisingly, there are two kinds of $LacI_4$+one iPTG complexes, as shown in the picture below. The seemingly two different $LacI_4$+one iPTG complexes on the left are identical to each other(rotating one around an axis in the plane of the paper will become the other) and are both denoted as $LacI_4$–1H. Similarly, the two on the right are identical to each other and denoted as $LacI_4$–1T.

There are four kinds of $LacI_4$+two iPTGs. The first two have both heads or both tails occupied. The complex with fully occupied heads is denoted as $LacI_4$-2HH, and the other is represented as $LacI_4$-2TT.



$LacI_4$+two iPTGs complexes could also have one head and one tail occupied. The two on the top both denoted as $LacI_2$-2HTtrans are identical and have the head of one dimer and the tail of another dimer occupied. The two on the bottom denoted as $LacI_2$-2HTgeminal identically have both the head and the tail from one dimer occupied.

Similar to $LacI_4$+one iPTG complexes, there are two $LacI_4$+three iPTGs complexes. In the figure below, two complexes on the left are identical and denoted as $LacI_4$-3HHT, and two complexes on the right are identical and denoted as $LacI_4$-3TTH.

Many species identified above are stereoisomers of each other. It is important to identify these different stereoisomers since they have different reactivity under the same conditions.

It is noteworthy to point out the implied stereospecificity of biochemical reactions and homochirality of proteins in this brief discussion of stereochemistry. iPTG happens to be a chiral molecule. If iPTG interacts with binding sites in only stereoselective but not stereospecific ways, then more pathways would exist and consequently more stereoisomers will emerge. The homochirality of proteins is also another obvious key factor in limiting the number of stereoisomers. The implications here are rather realistic since many biochemical processes involve the concept of "lock and key relationship" that strictly dictates the way molecules combine, and almost all biological molecules, such as protein, have the same configuration(or arguably conformation) that ensures their homochirality.

TetR and Tet are handled similarly. One key distinction between TetR and LacI is that TetR has no tetramers, only dimers.

5. Generation and Decay

The generation and decay of different species in *E Coli*. constitute two very important parts of the differential equation used to describe the concentration of one species.

5.1.1 Generation of Proteins

The way we characterize repressors like LacI without using Hill's Equation is to think of them as combining with the promoters—or more precisely, "promoter region", considering that LacI interacts with OLac but

not strictly the promoter—and therefore transforming the promoter region into a different form or complex.

Proteins are produced by ribosomes that read the mRNA sends out by RNAP reading DNA. Ribosomes and RNAPs are believed to both exist in large quantities in *E Coli*., and therefore the most important factor that limits protein production is the concentration and the intrinsic transcription-efficiency of different forms of promoter regions.

We describe such intrinsic transcription-efficiency of promoter regions with a variable η_{P_X}. It determines the rate of production of species Y by the P_X form of the promoter region in the following equation $\frac{d[Y]_{Generation}}{dt} = \eta_{P_X}[P_X]$. Such form of expression allows us to better include all kinds of promoter regions complexes varying contribution to the production of proteins—including constitutive expressions of repressors+promoter region complexes.

It is important to point out that until this point of discussion, the effect of RBS has not been considered. In this model, we arbitrarily exclude RBS from the vaguely defined term "Promoter Region" partially because we want to better investigate the influence RBS has on protein production. A new variable α_{RBS_Y} is added into the previous equation if Y is also regulated by an RBS, and the equation changes to $\frac{d[Y]_{Generation}}{dt} = \alpha_{RBS_Y}\eta_{P_X}[P_X]$.

It is necessary to further investigate the influence of our decision to exclude RBS from our vaguely defined term "Promoter Region".

Excluding RBS from the "Promoter Region" doesn't mean that RBS no longer plays an as important role in the production of proteins, instead, what it indicates is that RBS is more like a property of proteins and doesn't easily change.

In our previous example, if there is another "form of promoter region" called P_Z also controlling the production of Y, the production rate equation will then change to $\frac{d[Y]_{Generation}}{dt} = \alpha_{RBS_Y}(\eta_{P_X}[P_X] + \eta_{P_Z}[P_Z])$.

Since RBS is considered to be a property of proteins, the factor α_Y applies to both terms. We find this handling of RBS to be quite accurate. Although RBSs are easy to change during gene editing, it is rather stable afterward.

As in the previous model, the terminator "TT" is believed to totally terminating transcriptions.

5.1.2 Promoter Region Complexes and Stereochemistry

As indicated by our Figure 3, to associate with O_{Lac}, LacI dimers or tetramers must have the head and the tail from the same "dimer" to be "available", or not get struck by iPTG.

5.1.3 Generation of Promoter Regions

Promoter Regions are believed to be produced at some constant rate $\delta_{Replication}$, which is the rate for the replication of DNA and is thus applicable to all promoter regions.

5.2 Decay

Death or decay of various species is common. For a random species X, we characterize its rate of decay as a first-order reaction, $-k_X[X]$, with k_X as the rate constant regulating the rate of decay, and the minus sign indicating the diminish of the concentration of X.

6. iPTG and Diffusion

The effect of remaining *iPTG* on the engineering bacteria after tetracycline(Tet) is added to open up the production of Mf-Lon is re-evaluated. With our "death" line handling species with low concentrations, we can calculate the concentration of *iPTG* easily by taking the effect of *iPTG* passive transportation to the outside of the cell. Besides normal

7. Varying Types of Reactions and their Reversibility

Reactions are classified into a few basic types:
generation, decay, *iPTG* association, Tet association, symmetric association and dissociation, asymmetric association and dissociation, promoter region association and dissociation, and promoter regions complex transformation. Such classification is vital to have a deeper insight into the biochemical processes described.

7.1 Generation

As discussed above, see ***7. Production and Decay***. Generation either takes the form of a zeroth-order rate law or the form of a first-order rate law. Decay could be roughly considered as the reverse process of generation, though not that accurate.

For the production-rate-equation of a random species y, we have the following:

$$\frac{d[y]_{Generation}}{dt} = \begin{cases} +\delta_{\mathrm{Re}plication}, \ if \ y \ is \ a \ promoter \ region \\ +\alpha_{RBS_y}\left(\sum_{P_X}\eta_{P_X}[P_X]\right), \ if \ y \ is \ a \ protein \end{cases}.$$

7.2 Decay

As discussed above, see ***7, Production and Decay***. Decay takes the form of a zeroth-order rate law. Generation could be roughly considered as the reverse process of decay, though not that accurate.

For the decay-rate-equation of a random species y, we have the following:

$$\frac{d[y]_{Decay}}{dt} = -k_y[y].$$

7.3 iPTG Association

iPTG association is the process in which a LacI protein or LacI-iPTG complex combines an iPTG molecules. It is an irreversible second order reaction, since the attraction between LacI-proteins and iPTG is very strong.

The respective components of an iPTG association reaction $y + iPTG \rightarrow y-iPTG$ in the rate equations of y, iPTG, and $y-iPTG$ are

$$\begin{cases} \frac{d[y]_{iPTG-Association}}{dt} = -k_{y \to y-iPTG}[iPTG][y] \\ \frac{d[iPTG]_{iPTG-Association}}{dt} = -k_{y \to y-iPTG}[iPTG][y] \\ \frac{d[y-iPTG]_{iPTG-Association}}{dt} = +k_{y \to y-iPTG}[iPTG][y] \end{cases}$$, with $k_{y \to y-iPTG}$ being the rate constant of the reaction.

7.4 Tet Association

Similar to iPTG association, Tet association is the process in which a TetR protein or TetR-Tet complex combines an Tet molecules. It is an irreversible second order reaction, since the attraction between TetR-proteins and Tet is very strong.

The respective components of an Set association reaction $y + TetR \to y-TetR$ in the rate equations of y, TetR, and $y-Tet$ are

$$\begin{cases} \frac{d[y]_{Tet-Association}}{dt} = -k_{y \to y-Tet}[Tet][y] \\ \frac{d[Tet]_{Tet-Association}}{dt} = -k_{y \to y-Tet}[Tet][y] \\ \frac{d[y-Tet]_{Tet-Association}}{dt} = +k_{y \to y-Tet}[Tet][y] \end{cases}$$, with $k_{y \to y-Tet}$ being the rate constant of the reaction.

7.5 Symmetric Association and Dissociation

As indicated in its names, symmetric association and dissociation consists of two sub-processes—symmetric association and symmetric dissociation. Each sub-process is the reverse process of the other.

Symmetric association is the process of second rate law that involves two identical molecules combined to form a larger molecule, and symmetric association is the process of one large molecule broken down into two identical smaller molecule. These two sub-processes are closely interrelated and therefore handled together.

The respective components of one arbitrary symmetric association and dissociation $2y \underset{\text{and Dissociation}}{\overset{\text{Symmetric Association}}{\rightleftharpoons}} y_2$ on y and y_2 are

$$\begin{cases} \frac{d[y]_{\text{Symmetric Association and Dissociation}}}{dt} = -2k_{2y \rightleftharpoons y_2}[y]^2 + 2k_{2y \rightleftharpoons y_2}'[y_2] \\ \frac{d[y_2]_{\text{Symmetric Association and Dissociation}}}{dt} = -k_{2y \rightleftharpoons y_2}[y]^2 + k_{2y \rightleftharpoons y_2}'[y_2] \end{cases}$$, with

$k_{2y \rightleftharpoons y_2}$ being the rate constant for the forward reaction and $k_{2y \rightleftharpoons y_2}'$ being the rate constant for the reverse reaction.

7.6 Asymmetric Association and Dissociation

Asymmetric association and dissociation is only different from symmetric association and dissociation in the sense that asymmetric association and dissociation involves the "asymmetry"—being different from each other—instead of the "symmetry"—being same—of species combined in the association sub-process and the species produced in the dissociation sub-process.

The respective components of one arbitrary asymmetric association and dissociation $x + y \underset{\text{and Dissociation}}{\overset{\text{Asymmetric Association}}{\rightleftharpoons}} xy$ on x, y, and xy are

$$\begin{cases} \frac{d[x]}{dt} = -k_{x+y \rightleftharpoons xy}[x][y] + k_{x+y \rightleftharpoons xy}'[xy] \\ \frac{d[y]}{dt} = -k_{x+y \rightleftharpoons xy}[x][y] + k_{x+y \rightleftharpoons xy}'[xy] \\ \frac{d[xy]}{dt} = +k_{x+y \rightleftharpoons xy}[x][y] - k_{x+y \rightleftharpoons xy}'[xy] \end{cases},$$

in which $k_{x+y \rightleftharpoons xy}$ being the rate constant for the forward reaction and $k_{x+y \rightleftharpoons xy}'$ being the rate constant for the reverse reaction.

7.7 Promoter Region Association and Dissociation

Promoter region association is the process in which the promoter region combines with a molecule other than RNAP to form a complex. This process is regarded as irreversible.

Promoter region dissociation is the process in which the promoter region complex combines a small molecule like iPTG or Tet that deactivate the complex, leading to the dissociation of the promoter region complex. This process is regarded as irreversible as well.

We decide to merge these sub processes together into one category. Both sub-processes should be described with the second order rate law.

For a system consists of a promoter region P_X, a repressor or activator y, and a small molecule z that competes with the promoter region combining y, assume the following process exists: $P_X + y \xrightarrow[Association]{Promoter\ Region} P_X\text{-}y$ and $P_X\text{-}y + z \xrightarrow[Dissociation]{Promoter\ Region} P_X + yz$. Then the following equations apply:

$$\begin{cases} \frac{d[P_X]_{\text{Promoter Region Association and Dissociation}}}{dt} = -k_{P_X+y \to P_X-y}[P_X][y] + k_{P_X-y+z \to P_X+yz}[P_X-y][z] \\ \frac{d[y]_{\text{Promoter Region Association and Dissociation}}}{dt} = -k_{P_X+y \to P_X-y}[P_X][y] \\ \frac{d[P_X-y]_{\text{Promoter Region Association and Dissociation}}}{dt} = k_{P_X+y \to P_X-y}[P_X][y] - k_{P_X-y+z \to P_X+yz}[P_X-y][z] \\ \frac{d[z]_{\text{Promoter Region Association and Dissociation}}}{dt} = -k_{P_X-y+z \to P_X+yz}[P_X-y][z] \\ \frac{d[yz]_{\text{Promoter Region Association and Dissociation}}}{dt} = k_{P_X-y+z \to P_X+yz}[P_X-y][z] \end{cases}$$

, in which $k_{P_X+y \to P_X-y}$ is the rate constant for the association, and $k_{P_X-y+z \to P_X+yz}$ is the rate constant for the dissociation.

7.8 Promoter Region Complex Transformation

Promoter region complex transformation is the process in which the promoter region complex transforms into another promoter region complex in a one step second-order elementary reaction(vaguely defined).

Some transformations are reversible, such as a $LacI_2$-P_C complex combining with $LacI_2$ to form $LacI_4$-P_C. It is reversible because the connection between a $LacI_2$-P_C complex and a $LacI_2$ is considered to be similar in strength with the interactions between two LacI dimers, and since the symmetric association and dissociation of LacI dimers is reversible, this complex transformation should be reversible just as well.

Other transformations are irreversible. It is irreversible when a $LacI_4$-P_C interacts with iPTG to form $LacI_4$-1H-P_C since the interaction between a $LacI_4$-P_C complex and iPTG is considered to be nearly as strong as the interaction between $LacI_4$ and iPTG. Since iPTG addition is irreversible, this

process should be irreversible as well because the energy barrier for the reverse reaction is too high.

Although two kinds of complex transformations are handled slightly differently, they all follow kinetic equations.

A promoter region complex transformation $y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X$, has the following equations:

$$\begin{cases} \frac{d[y-P_X]}{dt} = -k_{y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X}[y-P_X][z] + k_{y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X}'[y-z-P_X] \\ \frac{d[z]}{dt} = -k_{y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X}[y-P_X][z] + k_{y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X}'[y-z-P_X] \\ \frac{d[y-z-P_X]}{dt} = k_{y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X}[y-P_X][z] - k_{y-P_X+z \underset{?}{\rightleftharpoons} y-z-P_X}'[y-z-P_X] \end{cases}.$$

If this transformation is reversible, then $k_{y-P_X+z \rightleftharpoons y-P_X+z}'$ is positive. If not, then $k_{y-P_X+z \rightleftharpoons y-P_X+z}'=0$.

8. All Possible Reactions

Decay and generation processes/reactions are not included here. With all the processes identified, we can easily build a system of differential equations based on ***9. Varying Types of Reactions and their Reversibility***.

8.1 Before iPTG is added

Symmetric Association and Dissociation

$$LacI \rightleftharpoons LacI_2 \rightleftharpoons LacI_4$$
$$TetR \rightleftharpoons TetR_2$$

Promoter Region Association and Dissociation

$$LacI_2 + P_C \rightarrow LacI_2 - P_C$$
$$LacI_4 + P_C \rightarrow LacI_4 - P_C$$
$$TetR_2 + P_M \rightarrow TetR_2 - P_M$$

Promoter Region Complex Transformation

$$LacI_2 - P_C + LacI_2 \rightleftharpoons LacI_4 - P_C$$

8.2 After iPTG is added

iPTG Association

$$LacI \rightarrow LacI-1$$
$$LacI_2 \rightarrow LacI_2-1H \text{ or } LacI_2-1T \rightarrow LacI_2-2$$
$$LacI_4 \rightarrow LacI_4-1H \text{ or } LacI_2-1T$$
$$LacI_4-1H \rightarrow LacI_4-2HH \text{ or } LacI_4-2HTtrans \text{ or } LacI_4-2HTgeminal$$
$$LacI_4-1T \rightarrow LacI_4-2TT \text{ or } LacI_4-2HTtrans \text{ or } LacI_4-2HTgeminal$$
$$LacI_4-2HH \rightarrow LacI_4-3HHT$$
$$LacI_4-2TT \rightarrow LacI_4-3TTH$$
$$LacI_4-2HTtrans \rightarrow LacI_4-3HHT \text{ or } LacI_4-3TTH$$
$$LacI_4-2HTgeminal \rightarrow LacI_4-3HHT \text{ or } LacI_4-3TTH$$
$$LacI_4-3HHT \rightarrow LacI_4-4$$
$$LacI_4-3TTH \rightarrow LacI_4-4$$

Tet Association

$$TetR_2 \rightarrow TetR_2-1H \text{ or } TetR_2-1T \rightarrow TetR_2-2$$

Symmetric Association and Dissociation

$$TetR \rightleftharpoons TetR_2$$
$$LacI \rightleftharpoons LacI_2 \rightleftharpoons LacI_4$$
$$LacI-1 \rightleftharpoons LacI_2-2 \rightleftharpoons LacI_4-4$$
$$LacI_2-1H \rightleftharpoons LacI_4-2HH$$
$$LacI_2-1T \rightleftharpoons LacI_4-2TT$$

Asymmetric Association and Dissociation

$$LacI+LacI-1 \rightleftharpoons LacI_2-1H \text{ or } LacI_2-1T$$
$$LacI_2+LacI_2-1H \rightleftharpoons LacI_4-1H$$
$$LacI_2+LacI_2-1T \rightleftharpoons LacI_4-1T$$
$$LacI_2+LacI_2-2 \rightleftharpoons LacI_4-2HTgeminal$$
$$LacI_2-1H+LacI_2-1T \rightleftharpoons LacI_4-2HTtrans$$
$$LacI_2-1H+LacI_2-2 \rightleftharpoons LacI_4-3HHT$$
$$LacI_2-1T+LacI_2-2 \rightleftharpoons LacI_4-3TTH$$

Promoter Region Association and Dissociation

$$TetR_2+P_M \rightarrow TetR_2-P_M$$
$$LacI_2+P_C \rightarrow LacI_2-P_C \rightarrow P_C+LacI_2-1H \text{ or } LacI_2-1T$$
$$LacI_4+P_C \rightarrow LacI_4-P_C \rightarrow P_C+LacI_4-1H \text{ or } LacI_4-1T$$
$$LacI_4-1H+P_C \rightarrow LacI_4-1H-P_C \rightarrow P_C+LacI_4-2HH \text{ or } LacI_4-2HTtrans$$
$$LacI_4-1T+P_C \rightarrow LacI_4-1T-P_C \rightarrow P_C+LacI_4-2TT \text{ or } LacI_4-2HTtrans$$
$$LacI_4-2HTgeminal+P_C \rightarrow LacI_4-2HTgeminal-P_C \rightarrow P_C+LacI_4-3HHT \text{ or } LacI_4-3TTH$$

Promoter Region Complex Transformation

$$LacI_2-P_C+LacI_2 \rightleftharpoons LacI_4-P_C$$
$$LacI_2-P_C+LacI_2-1H \rightleftharpoons LacI_4-1H-P_C$$
$$LacI_2-P_C+LacI_2-1T \rightleftharpoons LacI_4-1T-P_C$$
$$LacI_2-P_C+LacI_2-2 \rightleftharpoons LacI_4-2HTgeminal-P_C$$
$$LacI_4-P_C \rightarrow LacI_4-1H-P_C \rightarrow LacI_4-2HTgeminal-P_C$$
$$LacI_4-P_C \rightarrow LacI_4-1T-P_C \rightarrow LacI_4-2HTgeminal-P_C$$

8.3 After Tet is added

iPTG Association

$$LacI \rightarrow LacI-1$$
$$LacI_2 \rightarrow LacI_2-1H \text{ or } LacI_2-1T \rightarrow LacI_2-2$$
$$LacI_4 \rightarrow LacI_4-1H \text{ or } LacI_2-1T$$
$$LacI_4-1H \rightarrow LacI_4-2HH \text{ or } LacI_4-2HTtrans \text{ or } LacI_4-2HTgeminal$$
$$LacI_4-1T \rightarrow LacI_4-2TT \text{ or } LacI_4-2HTtrans \text{ or } LacI_4-2HTgeminal$$
$$LacI_4-2HH \rightarrow LacI_4-3HHT$$
$$LacI_4-2TT \rightarrow LacI_4-3TTH$$
$$LacI_4-2HTtrans \rightarrow LacI_4-3HHT \text{ or } LacI_4-3TTH$$
$$LacI_4-2HTgeminal \rightarrow LacI_4-3HHT \text{ or } LacI_4-3TTH$$
$$LacI_4-3HHT \rightarrow LacI_4-4$$
$$LacI_4-3TTH \rightarrow LacI_4-4$$

Tet Association

$$TetR \rightarrow TetR-1$$
$$TetR_2 \rightarrow TetR_2-1H \text{ or } TetR_2-1T \rightarrow TetR_2-2$$

Symmetric Association and Dissociation

$$TetR \rightleftharpoons TetR_2$$
$$TetR-1 \rightleftharpoons TetR_2-2$$
$$LacI \rightleftharpoons LacI_2 \rightleftharpoons LacI_4$$
$$LacI-1 \rightleftharpoons LacI_2-2 \rightleftharpoons LacI_4-4$$
$$LacI_2-1H \rightleftharpoons LacI_4-2HH$$
$$LacI_2-1T \rightleftharpoons LacI_4-2TT$$

Asymmetric Association and Dissociation

$$TetR+TetR-1 \rightleftharpoons TetR_2-1H \text{ or } TetR_2-1T$$
$$LacI+LacI-1 \rightleftharpoons LacI_2-1H \text{ or } LacI_2-1T$$
$$LacI_2+LacI_2-1H \rightleftharpoons LacI_4-1H$$
$$LacI_2+LacI_2-1T \rightleftharpoons LacI_4-1T$$
$$LacI_2+LacI_2-2 \rightleftharpoons LacI_4-2HTgeminal$$
$$LacI_2-1H+LacI_2-1T \rightleftharpoons LacI_4-2HTtrans$$
$$LacI_2-1H+LacI_2-2 \rightleftharpoons LacI_4-3HHT$$
$$LacI_2-1T+LacI_2-2 \rightleftharpoons LacI_4-3TTH$$

Promoter Region Association and Dissociation

$$TetR_2+P_M \rightarrow TetR_2-P_M \rightarrow P_M+TetR_2-1H \text{ or } TetR_2-1T$$
$$LacI_2+P_C \rightarrow LacI_2-P_C \rightarrow P_C+LacI_2-1H \text{ or } LacI_2-1T$$
$$LacI_4+P_C \rightarrow LacI_4-P_C \rightarrow P_C+LacI_4-1H \text{ or } LacI_4-1T$$
$$LacI_4-1H+P_C \rightarrow LacI_4-1H-P_C \rightarrow P_C+LacI_4-2HH \text{ or } LacI_4-2HTtrans$$
$$LacI_4-1T+P_C \rightarrow LacI_4-1T-P_C \rightarrow P_C+LacI_4-2TT \text{ or } LacI_4-2HTtrans$$
$$LacI_4-2HTgeminal+P_C \rightarrow LacI_4-2HTgeminal-P_C \rightarrow P_C+LacI_4-3HHT \text{ or } LacI_4-3TTH$$

Promoter Region Complex Transformation

$$LacI_2-P_C+LacI_2 \rightleftharpoons LacI_4-P_C$$
$$LacI_2-P_C+LacI_2-1H \rightleftharpoons LacI_4-1H-P_C$$
$$LacI_2-P_C+LacI_2-1T \rightleftharpoons LacI_4-1T-P_C$$
$$LacI_2-P_C+LacI_2-2 \rightleftharpoons LacI_4-2HTgeminal-P_C$$
$$LacI_4-P_C \rightarrow LacI_4-1H-P_C \rightarrow LacI_4-2HTgeminal-P_C$$
$$LacI_4-P_C \rightarrow LacI_4-1T-P_C \rightarrow LacI_4-2HTgeminal-P_C$$

9. The Statistical View

Before this point, all the equations we built and all the reactions we identified are considered to be ideal, or to say with a hundred percent efficiency. Nevertheless, this doesn't match our needs for the model—the predict the CAHS degradation time for an actual sample. An actual sample differs significantly from this "ideal-perfect-efficiency" cell in the sense that its intensity of life activities varies, and therefore an extra parameter $\eta_{Activity}$ should be included to represent the efficiency of different reactions and biochemical that is determined by the cell's life state. This parameter varies from cells to cells, and therefore makes the final degradation time we obtain to be of statistical importance, from which we can also determine the rough degradation time for a percentage of all samples to have CAHS degraded completely.

If some process is considered to be not influenced by the cell efficiency parameter, then in the rate expressions, the respective terms describing this process should be divided by $\eta_{Activity}$.

10. Cell Division

In our previous model, cell division is neglected. Nevertheless, our operational timescale is big enough to allow multiple cell divisions whose effect should better be included. We consider cells to stop all functions and biochemical processes during the process of cell division. One cell divines will produce two identical cells both having one half of the volume of the previous cell and the same concentration of every species. Therefore, the final result of a curve should be "broken up" with even intermittent flat lines to reflect this. All the equations we have right now have t as the independent variable. It represents the time before cell division is

considered, but it doesn't match our actual timescale. Our actual time scale is represented by

$$T = t + \alpha \eta_{Activity}{}^{2} b \left\lfloor \frac{t}{a} \right\rfloor$$

with a as the standard interval cell division time, b as the standard division time, $\eta_{Activity}$ squared because the actual interval cell division time and the standard division time are both regulated by $\eta_{Activity}$, and α as a constant.